Compound Identification from *Bromelia karatas* Fruit Juice Using Gas Chromatography–Mass Spectrometry and Evaluation of the Bactericidal Activity of the Extract

Benjamín A. Ayil-Gutiérrez 1, Karla Cecilia Amaya-Guardia 2, Arturo A. Alvarado-Segura 3, Glendy Polanco-Hernández 2, Miguel Angel Uc-Chuc 2, Karla Y. Acosta-Viana 2, Eugenia Guzmán-Marín 2, Blanca Yesenia Samaniego-Gámez 4, Wilberth Alfredo Poot-Poot 5, Gabriel Lizama-Uc 6 and Hernán de Jesús Villanueva-Alonso 7, 8

1 CONACYT—Instituto Politécnico Nacional, Centro de Biotecnología Genómica, Biotecnología Vegetal. Blvd. del Mtro, s/n, Esq. Elías Piña, Reynosa 88710, Mexico; bayil@ipn.mx
2 Laboratorio de Biología Celular, Centro de Investigaciones Regionales “Dr. Hideyo Noguchi”, Universidad Autónoma de Yucatán, Av. Itzáes, núm. 490 x calle 59, col. Centro, Mérida 97000, Mexico; karla.amaya@correo.uady.mx (K.C.-G.); glendy.polanco@correo.uady.mx (G.P-H.); ma.uc4outlook.com (M.A.U.-C.); aviana@correo.uady.mx (K.Y.-A.-V.); gmarin@correo.uady.mx (E.G.-M.)
3 Tecnológico Nacional México, Instituto Tecnológico Superior del Sur del Edo. de Yucatán. Carr. Muna-Felipe Carrillo Puerto, tramo Oskutzcab-Akil, Km. 41+400, Oskutzcab 97880, Mexico; aalvarado@surycatan.tecnm.mx
4 Facultad de Ingeniería y Ciencias, Instituto de Ciencias Agrícolas, Universidad Autónoma de Baja California, Carretera a Ejido Delta, Mexicali 21705, Mexico; samaniego.blanca@uabc.edu.mx
5 Laboratorio de Biotecnología Vegetal, Facultad de Ingeniería y Ciencias, Universidad Autónoma de Tamaulipas, Ciudad Victoria 87149, Mexico; waflaco@yahoo.com.mx
6 Tecnológico Nacional de México, Instituto Tecnológico de Mérida, Unidad de Posgrado e Investigación Av. Tecnológico, Km. 4.5 S/N, Mérida 97000, Mexico; gabriel.lu@merida.tecnm.mx
7 CONACYT—Laboratorio de Biología Celular, Centro de Investigaciones Regionales “Dr. Hideyo Noguchi”, Universidad Autónoma de Yucatán, Av. Itzáes, núm. 490 x calle 59, col. Centro, Mérida 97000, Mexico
8 Correspondence: herman.villanueva@correo.uady.mx; Tel.: +52-999-924-5755

Abstract: Fruits of species of the genus *Bromelia* contain compounds with health benefits and potential biotechnological applications. For example, *Bromelia karatas* fruits contain antioxidants and proteins with bactericidal activity, but studies regarding the activity of these metabolites and potential benefits are required. We evaluated the bactericidal activity of the methanolic extract (treated and not treated with activated charcoal) and its fractions (hexane, ethyl acetate, and methanol) from ripe *B. karatas* fruit (8 °Brix) against *Escherichia coli*, *Enterococcus faecalis*, *Salmonella enteritidis*, and *Shigella flexneri*. The methanolic extract (ME) minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined at eight concentrations. The methanolic extract MIC was 5 mg/mL for *E. faecalis* and 10 mg/mL for the other bacteria; the MBC was 20 mg/mL for *E. coli* and *E. faecalis*, and 40 mg/mL for *S. enteritidis* and *S. flexneri*. Through gas chromatography–mass spectrometry, 131 compounds were identified, some of which had previously been reported to have biological activities, such as bactericidal, fungicide, anticancer, anti-inflammatory, enzyme inhibiting, and anti-allergic properties. The most abundant compounds found in the ME of *B. karatas* fruits were maleic anhydride, 5-hydroxymethylfurfural, and itaconic anhydride. This study shows that *B. karatas* fruits contain metabolites that are potentially beneficial for health.

Keywords: bactericidal activity; *Bromelia karatas*; isocitrate lyase; itaconic anhydride; maleic anhydride

1. Introduction

Functional foods, which are consumed regularly as part of humans’ diets, can be defined as foods with bioactive components that, in addition to their impact on basic nutrition,
have been scientifically proven to reduce the risk of disease [1,2]. The consumption of fruits as functional foods and the evaluation of the different types of biological activities of their secondary metabolites have been increasing due to consumers’ awareness of the health benefits that they provide [3,4]. For example, there is evidence that for healthy women with recurrent urinary tract infections, the consumption of an optimal dose of cranberry extract can contribute to their prevention [5].

As Mexico is a megadiverse country with different vegetation types, it is home to a wide variety of wild fruits that are often used by one or more ethnic groups, such as the fruits of different species of bromeliads [6]. The genus *Bromelia* includes species that grow wild in Mexico and their fruits are known for their antifungal [7], bactericidal [6,8], and anthelmintic [9] activity. *Bromelia karatas* fruits contain antioxidants, proteases, and phenolic compounds, such as flavonoids, phenylpropanoids, terpenes, and coumarins [10,11]. Throughout tropical America, *B. karatas* fruits are consumed as foods and beverages, and their stems can be used as living fences. Traditional medicinal applications of this species include the treatment of helminth infections and certain types of ulcers [12,13]. It has been shown that prepurified proteases from *B. karatas* and *B. pinguin* fruits exhibited bactericidal activity against *Escherichia coli* and *Staphylococcus aureus*. However, it was shown that this activity is significantly reduced when the proteolytic extract is heated to 80 °C/15 min [14]. On the other hand, soluble protein extracts of *B. karatas* have been found to have a dose-dependent inhibitory effect on the growth of *Salmonella typhimurium* and *Listeria monocytogenes*, and proteolytic activity is suggested to play a role in the inhibition of *S. typhimurium* [15]. However, other studies regarding Bromeliads fruits have shown that methanolic extracts also have bactericidal activity [6].

This plant is a promising alternative and should be cultivated in tropical America and recognized as a functional food, thereby motivating the increase in its consumption. However, the specific compounds they contain and their biological activities that may benefit consumers’ health are not yet fully understood.

We evaluated bactericidal activity from a methanolic extract of *B. karatas* fruits and its fractions versus *Escherichia coli*, *Enterococcus faecalis*, *Salmonella enteritidis*, and *Shigella flexneri*. Finally, we identified compounds with previously reported biological activity that are potentially beneficial to the health of *B. karatas* fruit consumers.

### 2. Materials and Methods

#### 2.1. Fruit Collection and Juice Extraction

Ripe, wild *B. karatas* fruits were collected (Brix = 8 °Bx) during October near the town of Sitpach, in Merida, Yucatan, Mexico. The juice was extracted manually in the laboratory by cutting and squeezing the fruit. The pH of the juice was measured with an Extech ExStik®. The protein concentration was calculated following the Bradford method using the Quick StarTM Bradford Protein Assay commercial kit (Bio-Rad, Hercules, CA, USA), according to the manufacturer’s instructions.

#### 2.2. Antimicrobial Activity in Agar Well Diffusion

In a Petri dish with Mueller–Hinton II agar culture medium, 100 µL of a bacterial solution with turbidity equivalent to that of a 0.5 McFarland standard (1.5 × 10⁸ CFU/mL) was deposited, and with a sterile swab, the sample was distributed. Subsequently, 50 µL of the methanolic extract (50 mg/mL) was deposited in the wells (5 mm diameter). It was incubated for 24 h at 37 °C.

#### 2.3. Organic Extract

The organic extract was produced from 240 mL of juice. The juice was dried by placing it on a tray and heating it in an MS hybridization shaker oven (MO-AOR (Orbital)) at 50 °C for 24 h. Subsequently, the dried juice was pulverized, deposited in a 100 mL methanol flask, and left at room temperature. Methanol was replaced via filtration every 24 h for three days. Methanol from each of the three changes was collected in a flask and evaporated
to obtain the methanolic extract (ME). The methanolic extract was partitioned successively with hexane (HF), ethyl acetate (EAF), and methanol (MF) (Figure 1). The solvents were removed via evaporation and the fractions were dissolved in dimethyl sulfoxide (DMSO) at 0.5%.

**Figure 1.** Identification strategy for *Bromelia karatas* fruit compounds and their bactericidal activity evaluation.

### 2.4. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The microorganisms evaluated were *Escherichia coli* ATCC 35401, *Enterococcus faecalis*, *Salmonella enteritidis* ATCC 13076, and *Shigella flexneri* ATCC 12022. Both the MIC and MBC were determined using a 96-well plate. The four evaluated bacterial strains were cultured in Petri dishes on Mueller–Hinton medium at 37 °C for 16 h. One to two colonies were added to a test tube containing saline solution (0.85% NaCl) until turbidity equivalent to that of a 0.5 McFarland standard was reached (1.5 × 10^8 CFU/mL). This solution was diluted to 1.5 × 10^6 CFU/mL, and 50 µL was added to each well, followed by the addition of 50 µL of the solution (ME, EAF, or MF) to be tested at one of eight concentrations (0.625, 1.25, 2.5, 5, 10, 20, 40, or 80 mg/mL). In the case of synthetic metabolites (itaconic anhydride (ITAN), maleic anhydride (MA), and 5-hydroxymethylfurfural (5-HMF)), the concentrations were: 0.39, 0.78, 1.56, 3.125, 6.25, 12.5, and 25 mg/mL. The positive control was ampicillin, the negative control was bacteria alone, and the toxicity of 0.5% DMSO was evaluated. The 96-well plate was incubated at 37 °C for 18 h. The lowest concentration that did not present bacterial growth was taken as the MIC value. The MBC was measured by taking a sample from each well without turbidity, inoculating it in a Petri dish containing Mueller–Hinton medium, and incubating it at 37 °C for 18 h. The dish without observable bacterial growth was taken as the MBC.

### 2.5. Bactericidal Activity of the ME after Heating and Compound Removal with Activated Charcoal

Bactericidal thermostability was evaluated by heating 100 mg/mL of ME in an autoclave at 121 °C for 15 min, and then, bactericidal activity was measured through the agar well diffusion method. A second assay was performed to evaluate the bactericidal activity of the ME after treatment with activated charcoal (MEC). ME (100 mg) was dissolved in 1 mL of distilled water with 10% activated charcoal in a test tube. The mixture was vortexed for 5 min and centrifuged for 5 min at 12,000 g; bactericidal activity agar well diffusion was assessed using the supernatant. For further analysis, the MEC was dried by placing it on a tray and heating it in an MS hybridization shaker oven (MO-AOR (Orbital)) at 50 °C for 24 h.
2.6. Gas Chromatography–Mass Spectrometry of Organic Extract

The organic extract (30 mg extract/mL CH$_3$OH) was filtered through 0.2 µm nylon membranes (Thermo Scientific Cat. No. 726-2520). Then, 2 mL of the filtered solutions were injected into a gas chromatographer–mass spectrometer (GC-MS, Agilent 7890A) equipped with a hydrogen flame ionization detector for compound identification. Compound separation was conducted with an HP5MS column (30 m × 0.250 mm, 0.25 µm, Cat. No. 190915-435). The injector temperature was set at 250 °C and the initial oven temperature was 70 °C for 3 min, which increased at 5 °C/min to 250 °C.

2.7. Metabolites Quantification

High-performance liquid chromatography (HPLC) was used to determine the concentration of itaconic anhydride (ITAN), maleic anhydride (MA), and 5-hydroxymethylfurfural (5-HMF). Synthetic versions (Sigma-Aldrich, Saint Louis, MO, USA) of these three compounds were used as standards to make the calibration curve. The chromatographic system HPLC 1100 consisted of a quaternary system of pumps (Agilent Technologies G1310A, Waldbronn, Germany) connected to an automated sample injector (Agilent Technologies G1313A, Waldbronn, Germany). The compounds were detected using a wavelength of 210 nm (Agilent Technologies G1314A, Hachioji, Tokyo, Japan), and a reverse phase C18 column (Polaris 5 µm, 4.6 mm inner diameter × 250 Santa Clara, CA, USA). For the detection of ITA, MA, and 5-HMF, the mobile phase was H$_2$O/acetic acid at 0.05%. The flow rate for all of the samples was 0.625 mL/min and the column temperature was kept constant at 25 °C. The water used was previously degassed and filtered (ultrapure water; Milli-Q® ZMQS6V001).

2.8. Molecular Docking of the Mycobacterium tuberculosis Isocitrate Lyase (MtICL) with 5-HMF, ITAN, and MA

The crystal structure of the MtICL (PDB code = 6XPP) enzyme was used for docking analysis. The three-dimensional (3D) structure was downloaded from the Protein Data Bank database (https://www.rcsb.org/, accessed on 9 February 2022). The 3D structure was characterized and represented using X-ray crystallography [16].

A molecular docking experiment was conducted to evaluate the docking properties of four compounds (5-HMF, ITAN, and MA) with the MtICL enzyme. Docking was carried out with the HDOCK server (http://hdock.phys.hust.edu.cn/, accessed on 11 February 2022), a multi-component integrated package [17,18]. The molecular ligand formulas were downloaded from PubChem (https://pubchem.ncbi.nlm.nih.gov/, accessed on 11 February 2022), and a blind molecular docking grid box was used to define the docking region. The parameters were the default values in HDOCK, and the structures had a root mean square deviation (RMSD) of up to 3 Å. All of the results were analyzed and visualized using the UCSF Chimera 1.14 Molecular Graphics Systems [19].

3. Results

The ripe fruits collected had 8 °Bx; the average fruit weighed 19.2 g with 44% of juice, its pH was 3.25, and the protein content was 1870 mg/mL. The juice contained 5.8% ME, which exhibited bactericidal activity. This bactericidal activity remained unchanged after the ME was heat sterilized (at 121 °C, 15 min). The ME treated with 10% activated carbon generated an inhibition halo of 1.76 cm$^2$ in a bacterial culture, while the ME that did not undergo treatment generated an inhibition halo of 3.14 cm$^2$; this means that there was a 45% smaller inhibition halo when the ME was treated with activated charcoal.

The ME was fractionated using hexane (low polarity), ethyl acetate (medium polarity), and methanol (high polarity). It resulted in a 3% hexane fraction (FH), a 3% ethyl acetate fraction (EAF), a 68% methanol fraction (MF), and a 26% residue fraction (RF). The EAF and MF fractions exhibited bactericidal activity (Table 1).
Table 1. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of methanolic extract (ME) from *B. karatas* fruit, its fractions, and synthetic compounds (ITAN, MA, and 5-HMF).

| Bacteria          | E. coli | E. faecalis | S. enteritidis | S. flexneri |
|-------------------|---------|-------------|----------------|-------------|
| MIC (mg/mL)       |         |             |                |             |
| ME                | 10      | 5           | 10             | 10          |
| EAF               | 10      | 10          | 10             | 10          |
| MF                | 10      | 10          | 10             | 10          |
| ITAN              | 0.78    | 0.78        | 0.78           | 0.78        |
| MA                | 0.78    | 0.78        | 0.78           | 0.78        |
| 5-HMF             | 6.25    | 6.25        | 6.25           | 3.12        |
| MBC (mg/mL)       |         |             |                |             |
| ME                | 20      | 20          | 40             | 40          |
| EAF               | 20      | 20          | 20             | 20          |
| MF                | 20      | 20          | 20             | 20          |
| ITAN              | 1.56    | 1.56        | 1.56           | 1.56        |
| MA                | 1.56    | 1.56        | 1.56           | 1.56        |
| 5-HMF             | 12.5    | 12.5        | 12.5           | 6.25        |

To identify the metabolites that contributed to the antimicrobial activity and those with a possible biological activity that could affect fruit consumers, the methanolic extract (ME), methanolic extract treated with activated charcoal (MEC), and fractions with bactericidal activity (EAF and MF) were analyzed using the gas chromatography–mass (GC-MS) spectrometry (GS-MS) method. One hundred thirty-one compounds were identified, of which forty-nine were identified in the ME; in MEC, thirty-seven compounds were removed by activated charcoal and twenty-one new ones were detected (Table 2). On the other hand, in the ethyl acetate and methanolic fractions, compounds were detected which were not detected in the ME: 33 in EAF and 23 MF. Additionally, common and more abundant compounds in the ME, MEC, EAF, and MF were maleic anhydride (MA), 2,5 furandione, dihydro-3-methylene (itaconic anhydride; ITAN), and 5-hydroxymethylfurural (5-HMF) (Supplementary Material Tables S1–S4).

Table 2. Compounds detected in *Bromelia karatas* fruit juice extract.

| No. | Compound Name                  | Area % | No. | Compound Name                  | Area % |
|-----|--------------------------------|--------|-----|--------------------------------|--------|
| 1   | Maleic anhydride               | 18.98  | 26  | Thymine                        | 2.33   |
| *2  | 2-Methylpentyl formate          | 0.22   | 27  | 4H-Pyrane-4-one,2,3-dihydroxy-3,5-dihydroxy-6-methyl | 2.05   |
| *3  | 1-H-Imidazole, 4,5 dihydro-2-methyl | 0.28 | *28 | 2-Cyclopentene-1-one,3,5-hydroxy-2,3-dimethyl | 0.29   |
| *4  | Trans-2-Pentenoic acid         | 0.30   | *29 | Bicyclo [3.1.0] hexan -2-ol     | 0.20   |
| *5  | 1-H-Tetrazole 1-methyl         | 0.20   | *30 | Isobutyl nonyl carbonate       | 0.33   |
| *6  | N-(n-Butoxyethyl) acrylamide   | 0.26   | *31 | 2-Vinyl-9-[3-deoxy-beta-d-ribofuranosyl] hypoxanthine | 0.35   |
| *7  | 2-Heptanol, 5-ethyl            | 0.26   | 32  | 5-Hydroxymethylfurural         | 18.09  |
| 8   | Itaconic anhydride             | 22.47  | *33 | Thymol                         | 0.47   |
| 9   | 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one | 1.42 | *34 | 4-Hydroxy-3-methylacetophenone | 0.46   |
| *10 | 2H-Pyran, 3,4-dihydro          | 0.67   | *35 | 2-Methoxy-4-vinylphenol        | 0.81   |
| *11 | cis-1,4-Dimethylecyclohexane   | 0.56   | *36 | 3-Methoxycacetophenone         | 0.73   |
| 12  | 2 (3H)-Furanone                | 0.71   | *37 | 3,4-Diethylphenol              | 0.33   |
Table 2. Cont.

| No. | Compound Name                                  | Area % | No. | Compound Name                               | Area % |
|-----|-----------------------------------------------|--------|-----|---------------------------------------------|--------|
|13   | 2,5-Furandione, 3,4-dimethyl                  | 0.22   | *38 | Ethyl propionylacetate                      | 1.22   |
|*14  | Pent-2-ynal                                    | 0.29   | *39 | Methyl 3-hydroxypentanoate                  | 1.12   |
|*15  | 1,2-Butadiene                                  | 0.64   | *40 | 3-Methoxy-hexane-1,6-diol                    | 0.77   |
|16   | Hexan-3-yl acetate                             | 1.41   | *41 | Heptyl butyrate                              | 2.14   |
|*17  | Pentanoic acid, 4-oxo                         | 1.45   | *42 | Triethylene glycol monododecyl ether        | 1.21   |
|*18  | 1,5-Diacetoxypentane                           | 1.23   | *43 | Malic Acid                                   | 1.49   |
|*19  | 2-Propanamine, N-methyl-N-nitroso              | 1.72   | *44 | 2-Ethyl-3-hydroxyhexyl 2-methylpropanoate   | 1.37   |
|*20  | Methyl furan-3-carboxylate                     | 1.08   | *45 | 2,3,5,6-Tetrafluorobenzoic acid             | 0.60   |
|21   | Furly hydroxymethyl ketone                    | 1.26   | *46 | Ethanone, 1-(2,5-dimethoxyphenyl)            | 0.60   |
|22   | Methyl 2-furoate                               | 1.69   | *47 | 1,2,4-Cyclopentanetione, 3-(2-pentenyl)     | 0.40   |
|23   | Cyclopentene                                   | 1.11   | *48 | m-Ethyl aminophenol                          | 0.25   |
|*24  | 2-Cyclopentene-one, 2-methyl                   | 0.77   | *49 | 2,4,6-(3H)-Pteridinetrione, 1,5-dihydro     | 0.39   |
|*25  | 3H-Pyrazol-3-one, 2,4-dihydro-2,4,5-trimethyl  | 2.55   |     |                                             |        |

Note: Items with an asterisk were removed with activated charcoal treatment and aromatic compounds are underlined.

To evaluate whether common and more abundant compounds in the fractions and the ME contributed to the bactericidal activity, synthetic versions (Sigma-Aldrich) of them were tested, and they exhibited activity against all of the bacteria tested (Table 1). The concentrations of these three compounds in the extract and the fractions with bactericidal activity can be seen in Figure 2.

Figure 2. HPLC quantification of common compounds in the methanol extract (ME), the methanolic extract treated with activated charcoal (MEC), the ethyl acetate fraction (EAF), and the methanol fraction (MF).

4. Discussion

We determined the bactericidal activity of the *B. karatas* fruits’ methanolic extract (ME) against *E. coli*, *S. enteritidis*, *S. flexneri*, and *E. faecalis* (causal agents of human infections) [5,20]; it was shown that these results are consistent with those reported in Bromelia pinguin [6]. We found that the bactericidal activity resisted the sterilization temperature, meaning its potential benefits are still maintained when the fruits are consumed cooked since cooking is necessary to avoid oral injuries caused by their proteolytic activity [11]. In another study, it was determined that the protein extract of the fruits had bactericidal activity, but this was not resistant to heat [14,15], which differs from the heat-resistant bactericidal activity of the methanolic extract found in the present study.

The decrease of 45% in bactericidal activity after the treatment with activated charcoal can be explained by the ability of activated charcoal to remove compounds by adsorption, and the removal effectiveness is influenced by the molecular size, polarity, and branching of the molecule. For example, branched aromatic compounds are more effectively adsorbed on activated charcoal than linear and small molecules such as ethanol and methanol [21,22].
In the GC-MS analysis, 37 of the 49 compounds detected in the ME before charcoal treatment were removed, including 9 aromatic compounds. It was also possible to detect 26 compounds that were not detected in the ME; in this sense, activated charcoal is also likely to remove substances that were not detected in ME. The recovery of these removed compounds can be considered a method to isolate part of the antibacterial substances from B. Karatas fruits. However, the bactericidal activity of the substances removed still needs to be identified and evaluated.

On the other hand, the detection of new compounds in the MEC could have been because its concentration increased or the substances that prevented their detection, before treatment, were removed. In the same way, compounds were detected in the ethyl acetate and methanolic fractions that were not detected in the methanolic extract; this could be due to the compound enrichment and the removal of substances that interfere in the detection process [23]. Activated charcoal treatment and the partitioning of the methanolic extract into fractions of different polarities allowed for the broader detection of compounds.

The bactericidal activity level of the B. karatas fruits’ ME was too low (the MBC values were 20 mg/mL for E. coli and E. faecalis and 40 mg/mL for S. enteritidis and S. flexneri) for them to be considered sources of pure antibiotic activity [24]. However, being an edible fruit, the consumption of 10 fruits, with approximately 85 g of fruit juice (equivalent to 5 g of ME), may be enough to prevent mild bacterial infections, similar to the effect of consumption of cranberry fruit extracts [5,6,25]. However, this still needs to be evaluated.

In the GC-MS analysis of the extract and of the fractions with bactericidal activity (ME and MEC, EAF, and MF), we found that the common compounds were: maleic anhydride (MA), 2,5 furandione, dihydro-3-methylene (itaconic anhydride, ITAN), and 5-hydroxymethylfurfural (5-HMF). An increase in the concentration of 5-HMF in the MEC could be observed (Figure 2), possibly caused by the removal of other compounds; furthermore, 5-HMF could be produced during the drying process (50 °C/24 h) [26].

Maleic anhydride (MA) has been reported to be present in the copolymer manufacturing of biopolymers with activity against pathogens such as S. enteritidis, S. faecalis, and E. coli [27]. However, the results of the present study indicated that it exhibits bactericidal activity as a monomer, and 5-HMF is known to be an intermediary in the Maillard reaction, formed by the degradation of sugars at high temperatures. It has been found in various food products, including nuts, fruit juices, caramel products, coffee, bakery products, malt, and vinegar [28,29]. 5-HMF has anti-quorum sensing and anti-biofilm activity against Pseudomonas aeruginosa [30]. It is also reported to inhibit growth in E. coli LY01 [31], which is consistent with our results (Table 1). MA, ITAN, and 5-HMF showed activity against the bacteria evaluated (Table 1). However, their activity levels were relatively low [24] and their concentrations were not sufficient (Figure 2) to explain the ME activity against the bacteria analyzed. Therefore, further studies are required to identify the other bactericidal compounds of the ME.

We did not find evidence of the bactericidal activity of ITAN in the literature reports; however, it is reported to be an inhibitor of M. tuberculosis isocitrate lyase (MtICL) [32]. These authors showed that 0.03 mg/mL of ITAN can inhibit 90% of MtICL. This enzyme can promote the persistence and virulence of bacteria in macrophages, develop resistance to antibiotics, and promote the growth and survival of M. tuberculosis during its latent infection [33–35]. Considering their crucial roles, MtICLs are current inhibition targets for the development of new antibiotics to treat tuberculosis [35]. Considering that the structures of ITAN, MA, and 5-HMF have a certain degree of similarity, using molecular docking analysis, we evaluated whether MA and 5-HMF interact molecularly at the MtICL catalytic site as does ITAN. In Figure 3, it can be observed that MA, ITAN, and 5-HMF have similar binding patterns to the catalytic site. These results could suggest the possibility that MA and 5-HMF have roles in the inhibition of MtICL function. To fully understand this aspect, it is necessary to carry out enzyme activity assays; however, this was not the objective of this study.
Additionally, the ME was found to have activity against A. and K. C.A.-G.; software, M.A.U.-C.; formal analysis, B.A.A.-G. and H.d.J.V.-A.; writing—original Author Contributions: M. E. P.-H., E.G.-M., B.Y.S.-G., W.A.P.-P., G.L.-U.; supervision, H.d.J.V.-A. All authors have read and agreed to the published version of the manuscript.

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Table 3. Compounds with biological activity in Bromelia karatas juice.

| Compound                  | Biological Activity                                           | Reference |
|---------------------------|---------------------------------------------------------------|-----------|
| 2-Heptanol, 5-ethyl        | Possible alpha-amylase inhibitor.                             | [36]      |
| (2H)-Furanone              | It induces DNA damage and is possibly an anticancer.          | [37]      |
| Thymol                     | Bactericidal, fungicidal, anticarcinoma.                      | [38,39]   |
| 5-HMF                      | Inhibition of alcoholic hepatic oxidative injury, some       | [40-43]   |
|                           | toxicological effects, anti-inflammatory, and anti-allergic   |           |
|                           | effects.                                                      |           |
| 2-Methoxy-4-vinylphenol    | Anti-inflammatory and possible anticancer.                    | [44,45]   |
| 1,2,3-Benzenetriol         | anti-allergic                                                 | [46]      |

5. Conclusions

In this study, in the methanolic extract of B. karatas fruits, we identified the presence of several metabolites with a wide range of bioactivities, including bactericidal, fungicidal, anticancer, anti-inflammatory, enzyme inhibiting, and anti-allergic properties. Additionally, the ME was found to have activity against E. coli, E. faecalis, S. enteritidis, and S. flexneri. It was shown that the most abundant compounds of the ME are maleic anhydride, 5-hydroxymethylfurfural, and itaconic anhydride, which were active against the bacteria tested. This study shows the potential effects that B. karatas fruits could have on consumers’ health. However, more studies are still required.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/app12147275/s1, Table S1: Identified compounds in methanol extract; Table S2: Compounds identified in the methanolic extract treated with activated charcoal; Table S3: Compounds identified in the methanol fraction; Table S4: Compounds identified in the ethyl acetate fraction.

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