



Article

Influence of Extraction Technique and Solvent on the Recovery of Bioactive Compounds from *Eugenia uniflora* L. Fruits: Antioxidant, Antimicrobial, and UHPLC-MS/MS Characterization

Luana Gobo Pessanha ¹, Mariana Dalmagro ¹ , Larissa Aparecida Engel ¹, Talita Anielle de Assunção ² , Naira Neri Santana ³, Paula Derksen Macruz ⁴, Eduardo Jorge Pilau ⁴ , Evelyn Diane Pereira ¹ , Odair Alberton ^{1,3} , Emerson Luiz Botelho Lourenço ^{1,3,5} and Jaqueline Hoscheid ^{1,3,*}

- ¹ Postgraduate Program in Biotechnology Applied to Agriculture, University of Paraná, Umuarama 87502-210, Paraná, Brazil; luana.pes@edu.unipar.br (L.G.P.); mariana.dal@edu.unipar.br (M.D.); larissa.engel@edu.unipar.br (L.A.E.); evelyn.pibid@gmail.com (E.D.P.); odair@prof.unipar.br (O.A.); emerson@prof.unipar.br (E.L.B.L.)
- ² Pharmacy Academy, University of Paraná, Toledo 85903-170, Paraná, Brazil; talita.assuncao@edu.unipar.br
- ³ Professional Master's Program in Medicinal Plants and Herbal Medicines in Primary Care, University of Paraná, Umuarama 87502-210, Paraná, Brazil; naira51084@edu.unipar.br
- ⁴ Laboratory of Biomolecules and Mass Spectrometry, Department of Chemistry, State University of Maringá, Maringá 87080-000, Paraná, Brazil; pauladmacruz@gmail.com (P.D.M.); ejpilau@uem.br (E.J.P.)
- ⁵ Fundação Cândido Garcia, Umuarama 87502-210, Paraná, Brazil
- * Correspondence: jaqueline.hoscheid@gmail.com

Abstract

Fruits of *Eugenia uniflora* L. are rich in phenolic compounds and flavonoids, highlighting their potential as a source of bioactive compounds for nutraceutical development; however, extraction conditions can substantially influence the chemical composition and biological activity of the resulting extracts. This study evaluated the effects of extraction technique and solvent on the bioactive potential of *E. uniflora* fruit extracts. Powdered fruits were extracted by maceration, infusion, infusion followed by ethanol precipitation, and high-shear homogenization-assisted extraction (HSH) using water or 70% ethanol. Extracts were evaluated for yield, antioxidant capacity (DPPH, ABTS^{•+}, and FRAP), antimicrobial activity against six species, and chemical composition by UHPLC–MS/MS. HSH with 70% ethanol exhibited the highest DPPH (54.16 μmol Trolox equivalents g^{-1}) and FRAP (96.83 μmol Fe^{2+} g^{-1}) responses, whereas ethanolic maceration showed the highest ABTS^{•+} scavenging capacity (170.59 μmol Trolox equivalents g^{-1}). The ethanolic extracts showed greater antibacterial activity, particularly against *E. coli* (MIC = 6.51 μg mL^{-1}) and *S. epidermidis* (MIC = 15.63 μg mL^{-1}). UHPLC–MS/MS revealed diverse metabolites, with citric acid, quinic acid, and quercitrin showing prominent chromatographic peak areas, alongside other flavonoids, phenolic compounds, and organic acids. Overall, HSH with 70% ethanol provided the most balanced performance, supporting its potential as an efficient strategy for obtaining *E. uniflora* extracts with nutraceutical potential.

Keywords: flavonoids; high-shear homogenization-assisted extraction; nutraceuticals; pitanga



Academic Editor: Roberto Cannataro

Received: 14 August 2026

Revised: 14 September 2026

Accepted: 17 September 2026

Published: 2 October 2026

Copyright: © 2026 by the authors.

Licensee MDPI, Basel, Switzerland.

This article is an open access article distributed under the terms and

conditions of the [Creative Commons](#)

[Attribution \(CC BY\)](#) license.

1. Introduction

Natural products constitute important sources of bioactive molecules and chemical scaffolds for the development of functional ingredients, nutraceuticals, and other value-added products [1,2]. The interest in plant-derived substances is associated with the search for raw materials that combine bioactive constituents and functional properties with production processes that are more compatible with sustainability principles. In this context, fruits rich in phenolic compounds, flavonoids, natural pigments, vitamins, and other specialized metabolites are of particular relevance to the food and dietary supplement industries [2].

Among native Brazilian species with functional potential, *Eugenia uniflora* L. (Myrtaceae), commonly known as pitanga, stands out. Its fruits are consumed fresh and used in the production of juices, jellies, pulps, and other derivatives. Pitanga exhibits a diverse chemical composition, including phenolic compounds, flavonoids, tannins, anthocyanins, carotenoids, vitamins, and organic acids, whose distribution may vary according to variety, maturation stage, cultivation conditions, and the plant part evaluated [3–6]. This chemical diversity has been associated with antioxidant, antimicrobial, and metabolic activities, reinforcing the potential of this species as a source of value-added natural ingredients [3,6].

Despite the recognized potential of this species, a substantial proportion of studies on *E. uniflora* has focused on its leaves, whereas its fruits remain comparatively underexplored from technological and chemical perspectives [3,6,7]. As an edible matrix, the fruit is of particular interest for the development of ingredients with nutraceutical potential. However, such applications depend on obtaining reproducible extracts with characterized chemical composition and demonstrable biological activity [2]. In this regard, the selection of the extraction procedure represents a critical step, as differences in solvent polarity, temperature, contact time, and mechanical disruption intensity may affect both overall extraction yield and the selective recovery of bioactive metabolites [7].

Traditional methods, such as infusion and maceration, are widely employed for obtaining plant extracts because of their operational simplicity and low cost [2]. However, these procedures may require prolonged contact times and provide less control over mass transfer. Infusion followed by ethanol precipitation (EPInf), in turn, may remove proteins, polysaccharides, and other higher-molecular-weight constituents, thereby modifying the composition of the final extract. In contrast, techniques based on high-shear homogenization, such as high-shear homogenization-assisted extraction (HSH), promote intense physical disruption of the plant matrix through rotor–stator action, increasing the contact area between the material and solvent and favoring the release of intracellular constituents within shorter processing times [8–10].

In addition to the extraction technique, solvent selection plays a central role in extraction selectivity. Water exhibits high polarity and is compatible with food applications, making it particularly suitable for the recovery of hydrophilic constituents. Ethanol, especially when used in hydroethanolic mixtures, has intermediate polarity and can broaden the range of solubilized compounds, including different classes of phenolics and flavonoids [2,7,10]. However, solvent efficiency cannot be considered independently, as it results from the interaction among solvent properties, extraction technique, matrix structure, and the physicochemical characteristics of the target compounds [7].

Regular consumption of antioxidant compounds has been associated with the mitigation of oxidative stress, which is linked to the development and progression of chronic noncommunicable diseases, including cardiovascular, neurodegenerative, and metabolic disorders, as well as inflammatory processes [6,11]. In this context, standardized plant extracts have attracted increasing interest in the nutraceutical field because they may pro-

vide more consistent concentrations of bioactive compounds, potentially supporting the prevention of oxidative damage and promoting health [6].

However, the potential efficacy of such nutraceutical ingredients depends on the preservation and recovery of antioxidant compounds during extract preparation. Therefore, efficient extraction technologies are essential to maximize the recovery of bioactive molecules while preserving their chemical stability and biological activity [8].

Although the bioactive potential of *E. uniflora* fruits has been previously described, studies comparing traditional extraction procedures and HSH in an integrated manner remain limited, particularly those simultaneously considering extraction yield, different antioxidant mechanisms, antimicrobial activity, and chemical profile. Moreover, the selection of a promising condition for nutraceutical applications requires consideration of the overall response profile rather than a single performance parameter.

Therefore, this study aimed to evaluate the influence of different extraction procedures on the yield, antioxidant capacity, antimicrobial activity, and chemical profile of *E. uniflora* fruit extracts, with the objective of identifying the most promising condition for obtaining an ingredient with nutraceutical potential.

2. Materials and Methods

2.1. Plant Material and Sample Preparation

Ripe fruits of *Eugenia uniflora* (red pitanga variety) were collected in September 2025 in the municipality of Umuarama, Paraná, Brazil (23°46′09.1″ S, 53°16′38.4″ W). A voucher specimen was deposited in the Herbarium of Universidade Paranaense under registration number 12. Access to the genetic heritage was registered in the Brazilian National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SisGen) under protocol number A625A5A.

The ripe fruit pulps were manually separated from the seeds, frozen, and freeze-dried. The lyophilized material was ground using a knife mill and subjected to particle size analysis using a series of standard Tyler sieves. The fraction with a particle size of 0.53 mm was selected and used for the extraction procedures.

2.2. Extraction Procedures

The extracts were prepared using distilled water or 70% ethanol (Dinâmica, Indaiatuba, Brazil) as extraction solvents. A 1:10 (*w/v*) ratio was maintained between the lyophilized plant material and the extraction solvent. The use of 70% ethanol was selected based on previous reports of its application for the extraction of bioactive constituents from *E. uniflora* fruits [12].

After each extraction procedure, the samples were subjected to vacuum filtration. Ethanol present in the hydroethanolic extracts was removed under reduced pressure using a rotary evaporator (Nova Ética, Vargem Grande Paulista, Brazil) at 45 °C and −630 mmHg, and all extracts were subsequently freeze-dried (JJ Científica, model LJJ02, São Paulo, Brazil) at −42 °C and 0.10 mbar, and stored at −20 °C until analysis. Each extraction procedure was performed using three independent preparations.

2.2.1. Infusion

The infusion was prepared according to the procedure described by Pinc et al. [13] for fruits of another species belonging to the family Myrtaceae. Briefly, distilled water previously heated to 90 °C was added to the lyophilized plant material. The container was immediately closed and left to stand for 6 h until the preparation reached room temperature. The sample was then vacuum-filtered, and the resulting extract was freeze-dried.

2.2.2. Infusion Followed by Ethanol Precipitation (EPInf)

Initially, the infusion was prepared as described in the previous section. After filtration, 99.5% ethanol (Dinâmica, Indaiatuba, Brazil) was added at a 1:3 (*v/v*) infusion-to-ethanol ratio. The mixture was left to stand for 48 h to promote the precipitation of proteins, polysaccharides, and other higher-molecular-weight constituents. After this period, the preparation was filtered again [13]. The recovered fraction was subjected to ethanol removal under reduced pressure and subsequently freeze-dried.

2.2.3. Maceration

The plant material was placed in contact with 70% ethanol in a hermetically sealed container, protected from light, and maintained at room temperature for seven days without solvent renewal. After the extraction period, the samples were filtered and concentrated to dryness. The maceration procedure was performed according to the method described by Tominc et al. [14].

2.2.4. High-Shear Homogenization-Assisted Extraction

HSH was performed using a digital Ultra-Turrax® T25 disperser equipped with an 18G dispersing element (IKA, Staufen, Germany). The plant material was separately extracted with distilled water and 70% ethanol, maintaining a 1:10 (*w/v*) sample-to-solvent ratio. Extractions were performed at 12,000 rpm for 10 min at 20 °C using a jacketed extraction vessel connected to a thermostatic water bath set at 20 °C, which was used to maintain the extraction temperature throughout the homogenization process. The HSH procedure was based on a previously reported method by Teixeira et al. [15], with modifications and adjustments established following preliminary tests conducted with pitanga fruit material. Immediately after extraction, the samples were vacuum-filtered. The ethanolic extract was subjected to solvent removal under reduced pressure, after which both extracts were freeze-dried.

2.3. Extraction Yield

Extraction yield was determined gravimetrically based on the mass of the dry extract obtained after freeze-drying and the initial mass of lyophilized plant material used for each extraction. Results were expressed as the percentage of dry extract relative to the initial mass of plant material on a dry-weight basis.

2.4. Antioxidant Capacity

The antioxidant capacity of the *E. uniflora* fruit extracts was evaluated using the ferric reducing antioxidant power (FRAP), ABTS•⁺ (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) radical-scavenging, and DPPH (2,2-Diphenyl-1-picrylhydrazyl) radical-scavenging assays. Extracts were dissolved at a concentration of 1000 µg mL⁻¹, and each extract was analyzed in three technical replicates.

For the FRAP assay, 90 µL of each extract solution was added to 270 µL of distilled water and 2.7 mL of freshly prepared FRAP reagent (300 mM acetate buffer, 10 mM TPTZ (purity ≥ 98.0%; Sigma-Aldrich, St. Louis, MO, USA), and 20 mM FeCl₃ (purity ≥ 97.0%; Sigma-Aldrich, St. Louis, MO, USA)). The reaction mixture was incubated at 37 °C for 30 min, and the absorbance was measured at 595 nm using a UV-Vis spectrophotometer (Kasuki, Model IL-592, São Paulo, Brazil). Quantification was performed using a ferrous sulfate (purity ≥ 99.0%; Sigma-Aldrich, St. Louis, MO, USA) calibration curve ranging from 100 to 2000 µM. The results were expressed as µmol Fe²⁺ per gram of dry extract (µmol Fe²⁺ g⁻¹), according to Benzie and Strain [16].

For the ABTS^{•+} assay, 10 µL of each extract solution was added to 1.0 mL of ABTS^{•+} (purity ≥ 98.0%; Sigma-Aldrich, St. Louis, MO, USA) solution. After homogenization, the mixtures were incubated for 6 min at 25 °C, and the absorbance was measured at 734 nm. Ethanol was used as the blank. Quantification was performed using a Trolox (purity ≥ 97.0%; Sigma-Aldrich, St. Louis, MO, USA) calibration curve ranging from 100 to 2000 µM. The results were expressed as µmol Trolox equivalents per gram of dry extract (µmol TE g⁻¹), according to Re et al. [17].

For the DPPH assay, 150 µL of each extract solution was mixed with 5.850 mL of an ethanolic DPPH solution (0.06 mM; purity ≥ 97.0%; Sigma-Aldrich, St. Louis, MO, USA). The mixtures were incubated for 30 min at 25 °C, and the absorbance was measured at 515 nm. Ethanol was used as the blank. Quantification was performed using a Trolox calibration curve ranging from 50 to 1000 µM. The results were expressed as µmol Trolox equivalents per gram of dry extract (µmol TE g⁻¹), according to Brand-Williams et al. [18].

2.5. Antimicrobial Activity

The potential antimicrobial activity was evaluated against the strains *Staphylococcus aureus* CCCD-S007, *Staphylococcus epidermidis* CCCD-S010, *Escherichia coli* CCCD-E003, *Pseudomonas aeruginosa* CCCD-1004, *Salmonella enterica* subsp. *enterica* CCCD-50016, and the yeast *Candida albicans* CCCD-CC001, all obtained from Cefar Diagnóstica® (São Paulo, Brazil).

The minimum inhibitory concentration (MIC) was determined using the broth microdilution method in 96-well microplates, according to the recommendations of the Clinical and Laboratory Standards Institute [19].

For bacterial assays, 100 µL of Brain Heart Infusion (BHI, Acumedia®, Lansing, MI, USA) broth was added to each well, whereas 100 µL of Sabouraud dextrose broth (Acumedia®, Lansing, MI, USA) was used for *C. albicans*. Starting with the second column, 100 µL of the extract stock solution, prepared at a concentration of 1000 µg mL⁻¹, was added. Serial two-fold dilutions were subsequently performed through the 11th column, resulting in final extract concentrations ranging from 0.976 to 500 µg mL⁻¹.

Microbial suspensions were previously standardized to 0.5 McFarland turbidity, corresponding to approximately 1.5 × 10⁸ colony-forming units (CFU) mL⁻¹ for bacteria and 1.5 × 10⁶ CFU mL⁻¹ for the yeast. Subsequently, 5 µL of each microbial suspension, corresponding to approximately 7.5 × 10⁵ CFU for bacteria and 7.5 × 10³ CFU for yeast, were added to the corresponding wells.

The first column served as the growth control and contained the culture medium and microbial inoculum without extract. A reference antimicrobial control was included using a neomycin solution prepared at 1000 µg mL⁻¹, and its MIC was determined under the same assay conditions. Vehicle controls containing the respective extraction solvent, without extract, and extract blanks containing the corresponding extract and culture medium, but without microbial inoculum, were included in the last row of the microplate to assess any potential antimicrobial effect of the solvents and to account for possible interference from the extracts, respectively. The 12th column served as the sterility control and contained the culture medium without microbial inoculum.

The microplates containing bacterial cultures were incubated at 36 °C for 24 h. After incubation, 20 µL of a 2% solution of 2,3,5-triphenyltetrazolium chloride (purity ≥ 99.0%; Êxodo Científica, Sumaré, Brazil) was added to each well, followed by an additional 2 h incubation. Development of a red coloration was considered indicative of metabolic activity and bacterial growth, whereas the absence of coloration indicated growth inhibition.

The microplates containing *C. albicans* were incubated at 27 °C for 48 h. Yeast growth was assessed visually based on the presence of turbidity in the wells. The MIC was defined

as the lowest extract concentration, expressed in $\mu\text{g mL}^{-1}$, capable of preventing visible microbial growth. Each extract was analyzed in three technical replicates.

2.6. Chemical Profiling by UHPLC–MS/MS

Pitanga extracts were analyzed using a UHPLC–MS/MS system (Shimadzu Nexera X2, Kyoto, Japan) coupled to a Q-TOF Impact II mass spectrometer (Bruker, Billerica, MA, USA). Three independent extraction replicates were prepared for each extraction procedure and pooled prior to UHPLC–MS/MS analysis to obtain a representative sample for each treatment. Approximately 10 mg of each pooled sample was dissolved in 1 mL of MeOH:H₂O (7:3, *v/v*), vortexed for 5 min, centrifuged for 5 min, and filtered through polytetrafluoroethylene (PTFE) filters (Millex, SLLGX13, 0.22 $\mu\text{m} \times 13 \text{ mm}$, Millipore, Burlington, MA, USA). Each pooled extract was analyzed in triplicate. The injection volume was 2 μL .

Chromatographic separation was performed using a CSH C18 column (Waters Corporation, Milford, MA, USA; 1.7 μm , 2.1 $\times$ 100 mm) maintained at 40 °C. The mobile phase consisted of ultrapure water containing 0.1% formic acid (phase A) and methanol (phase B) for negative ionization mode, and ultrapure water containing 0.1% formic acid (phase A) and methanol containing 0.1% formic acid (phase B) for positive ionization mode. All solvents were LC–MS hypergrade (purity $\geq 99.9\%$; Sulpeco, Darmstadt, Germany). Separation was performed over 20 min using the following gradient: 0.1 min, 10% B; 0.1–7 min, 50% B; 7–12 min, 98% B; 12–15 min, 98% B; 15–17 min, 10% B; and 17–20 min, 10% B. The flow rate was maintained at 0.250 mL min^{−1} throughout the chromatographic run.

The Q-TOF Impact II mass spectrometer equipped with an electrospray ionization (ESI) source was operated in automatic MS/MS acquisition mode. The acquisition rate was 5 Hz for both MS and MS/MS, with an *m/z* range of 120–1200. Mass spectra were acquired in both positive and negative ionization modes, with the capillary voltage set to 3.50 kV, source temperature maintained at 200 °C, and desolvation gas flow rate set to 9 L min^{−1}.

Ion chromatograms and MS and MS/MS spectra were processed and visualized using Data Analysis 4.3 software (Bruker, Billerica, MA, USA). Metabolites were putatively annotated by comparing their accurate masses and MS/MS fragmentation spectra with those available in the MassBank of North America (MoNA) database and with data previously reported in the literature. Only signals with a mass error lower than 10 ppm were considered for annotation. Because authentic standards were not analyzed, the annotated metabolites were classified as level 2 (putatively annotated compounds) according to the Metabolomics Standards Initiative (MSI) criteria proposed by Sumner et al. [20]. The relative abundance of the annotated metabolites was calculated from their chromatographic peak areas and expressed as a percentage of the total chromatographic peak area, providing a semi-quantitative comparison among the extracts.

2.7. Statistical Analysis

The extraction procedures and antioxidant and antimicrobial activity assays were conducted using a completely randomized design. Each treatment consisted of three independent preparations, and the results were expressed as mean $\pm$ standard deviation. The data were subjected to analysis of variance (ANOVA). When significant differences were detected, means were compared and grouped using the Scott–Knott test at a 5% significance level. Statistical analyses were performed using Statistica software, version 13.0 (StatSoft, Inc., Tulsa, OK, USA).

3. Results

The aqueous extracts exhibited a light yellow to orange, opaque appearance and a viscous, slightly sticky consistency. In contrast, the extracts obtained with 70% ethanol showed an intense red coloration and a dry appearance. These differences in visual characteristics were consistently observed among the extracts obtained with the different solvents.

Extraction yields differed significantly among the procedures, ranging from 17.77% to 57.41%. HSH with 70% ethanol produced the highest extraction yield, although it did not differ statistically from ethanolic maceration (Table 1). The lowest yield was obtained by HSH with water, which differed significantly from all other treatments.

Table 1. Extraction yields of *E. uniflora* fruit extracts obtained using different extraction procedures.

Extractive Process	Solvent	Yield (%)
Infusion	Water	50.07 ± 3.89 ^b
EPInf	Water	36.84 ± 1.63 ^c
Maceration	70% ethanol	55.70 ± 2.74 ^{ab}
HSH	Water	17.77 ± 1.30 ^d
HSH	70% ethanol	57.41 ± 1.21 ^a

Note: EPInf—infusion followed by ethanol precipitation; HSH—high-shear homogenization-assisted extraction. Data are presented as mean ± standard deviation (SD) of three independent extractions. Means followed by the same letter are not significantly different according to the Scott–Knott test ($p \leq 0.05$).

Antioxidant capacity varied significantly according to both the extraction procedure and the analytical method employed (Table 2). HSH with 70% ethanol yielded the highest antioxidant capacity in the FRAP and DPPH assays, whereas ethanolic maceration exhibited the highest ABTS^{•+} scavenging capacity. Overall, extracts prepared with water showed lower antioxidant responses than those obtained with 70% ethanol.

Table 2. Antioxidant capacity of *E. uniflora* fruit extracts obtained using different extraction procedures.

Extractive Process	Solvent	FRAP	ABTS ^{•+}	DPPH
Infusion	Water	83.57 ± 0.00 ^b	125.22 ± 8.02 ^b	3.96 ± 5.00 ^b
EPInf	Water	67.82 ± 8.21 ^c	104.42 ± 0.00 ^c	nd
Maceration	70% ethanol	84.40 ± 1.17 ^b	170.59 ± 8.02 ^a	nd
HSH	Water	62.02 ± 2.34 ^c	123.33 ± 10.69 ^b	nd
HSH	70% ethanol	96.83 ± 0.00 ^a	119.55 ± 16.04 ^b	54.16 ± 10.58 ^a

Note: EPInf—infusion followed by ethanol precipitation; HSH—high-shear homogenization-assisted extraction; FRAP—ferric reducing antioxidant power, expressed as $\mu\text{mol Fe}^{2+} \text{ g}^{-1}$ dry extract; ABTS^{•+} and DPPH—radical-scavenging capacity expressed as $\mu\text{mol Trolox equivalents (TE)} \text{ g}^{-1}$ dry extract; nd—no detectable DPPH activity. Different letters within the same column indicate statistically significant differences according to the Scott–Knott test ($p \leq 0.05$).

The antimicrobial activity of *E. uniflora* fruit extracts varied according to both the extraction procedure and the microorganism evaluated. Overall, lower MIC values were observed for extracts obtained with 70% ethanol, particularly those prepared by HSH, indicating greater inhibitory activity under the conditions tested (Table 3), especially against *E. coli*, *S. epidermidis*, *P. aeruginosa*, and *S. enterica* subsp. *enterica*.

In contrast, HSH with water showed no inhibitory activity against any of the microorganisms under the conditions evaluated. Likewise, none of the extracts exhibited activity against the fungus *C. albicans*.

The chemical profiling of *E. uniflora* fruit extracts allowed the annotation of 22 signals in positive ionization mode (Table 4) and 24 signals in negative ionization mode (Table 5). Different metabolite classes were identified, including organic acids, phenolic acids, free and glycosylated flavonoids, fatty acids, and terpenoids.

The five extracts shared a common chemical profile, as the annotated signals were detected across all extraction procedures. However, the chromatographic areas of individual metabolites varied among extracts, indicating descriptive differences in the relative recovery of constituents according to the extraction conditions. Among the annotated compounds, citric acid, quinic acid, and quercitrin were particularly prominent.

Direct comparison of the two HSH extracts showed that replacing water with 70% ethanol was associated with greater chromatographic areas for several glycosylated flavonoids, particularly myricetin and quercetin derivatives. In contrast, the aqueous extract exhibited greater areas for some organic acids.

Table 3. Minimum inhibitory concentrations of *E. uniflora* fruit extracts against the microorganisms evaluated.

Extractive Process	<i>S. aureus</i>	<i>S. epidermidis</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>C. albicans</i>	<i>S. enterica</i>
Infusion	125.00 ± 0.00	>500	62.50 ± 0.00	250.00 ± 0.00	>500	>500
EPInf	250.00 ± 0.00	250.00 ± 0.00	194.44 ± 59.43	250.00 ± 0.00	>500	62.50 ± 0.00
Maceration	250.00 ± 0.00	62.50 ± 0.00	31.25 ± 0.00	250.00 ± 0.00	>500	62.50 ± 0.00
HSH—water	>500	>500	>500	>500	>500	>500
HSH—ethanol	125.00 ± 0.00	15.63 ± 0.00	6.51 ± 1.95	104.17 ± 30.41	>500	31.25 ± 0.00
Neomycin	$6.50 \times 10^{-3} \pm 0.00$	$2.28 \times 10^{-3} \pm 0.00$	$1.30 \times 10^{-3} \pm 0.00$	$1.56 \times 10^{-2} \pm 0.00$	>500	$8.30 \times 10^{-2} \pm 0.03$

Note: EPInf—infusion followed by ethanol precipitation; HSH—high-shear homogenization-assisted extraction. The symbol > 500 indicates no inhibition at the highest concentration tested. For *C. albicans*, neomycin did not exhibit inhibitory activity at concentrations up to 500 µg mL^{−1}; therefore, the absence of a specific antifungal reference control should be considered when interpreting the antifungal results. Values are expressed as µg mL^{−1} and presented as mean ± standard deviation of three independent preparations. The MIC corresponds to the lowest extract concentration capable of preventing visible microbial growth. Lower MIC values indicate greater inhibitory activity.

Table 4. Identification of the constituents of *E. uniflora* extracts using ultra-high-performance liquid chromatography–tandem mass spectrometry (UHPLC–MS/MS) in positive mode.

Compound	RT	Molecular Formula	Theoretical <i>m/z</i>	Experimental <i>m/z</i> **	Relative Abundance (%) *				
					Inf	EPInf	Mac	HSH Water	HSH Ethanol
Quinic acid	1.69	C ₇ H ₁₂ O ₆	193.0706	193.0694	30.53	21.40	12.87	20.22	16.31
Epigallocatechin	2.24	C ₁₅ H ₁₄ O ₇	307.0812	307.0807	4.17	3.95	2.01	3.84	2.38
Citric acid	2.48	C ₆ H ₈ O ₇	193.0342	193.0325	30.84	36.11	22.73	39.02	23.69
Chlorogenic acid	6.45	C ₁₆ H ₁₈ O ₉	355.1023	355.1006	1.04	1.47	0.64	1.66	1.41
12-hydroxyjasmonic acid	7.35	C ₁₂ H ₁₈ O ₄	227.1277	227.1260	0.68	0.73	0.42	1.27	0.40
Myricetin O-pentoside	7.64	C ₂₀ H ₁₈ O ₁₂	451.0871	451.0847	0.05	0.08	0.63	0.04	0.83
Myricitrin (Isomer I)	7.72	C ₂₁ H ₂₀ O ₁₂	465.1027	465.1001	0.12	0.18	0.99	0.12	1.32
Myricetin-3-Galactoside	8.09	C ₂₁ H ₂₀ O ₁₃	481.0976	481.0953	0.37	0.39	1.33	0.25	1.62
Loliolide	8.15	C ₁₁ H ₁₆ O ₃	197.1170	197.1159	1.53	1.81	0.28	0.11	0.06
2''-O-Galloylhyperin	8.45	C ₂₈ H ₂₄ O ₁₆	617.1129	617.1098	0.73	0.85	1.91	0.80	2.07
Myricitrin (Isomer II)	8.71	C ₂₁ H ₂₀ O ₁₂	465.1027	465.1012	1.36	1.38	2.94	0.61	3.19
Myricetin	8.71	C ₁₅ H ₁₀ O ₈	319.0455	319.0439	1.74	1.83	4.17	0.81	4.67
Isoquercetin	9.02	C ₂₁ H ₂₀ O ₁₂	465.1028	465.0999	5.64	6.49	10.49	5.21	10.86
Quercetin O-pentoside	9.45	C ₂₀ H ₁₈ O ₁₁	435.0910	435.0908	4.21	4.78	9.89	3.53	10.49
Kaempferol-3-glucoside	9.58	C ₂₁ H ₂₀ O ₁₁	449.1077	449.1043	0.49	0.73	1.03	0.63	1.07
Quercitrin	9.71	C ₂₁ H ₂₀ O ₁₁	449.1077	449.1051	9.94	10.41	12.09	10.16	12.40

Table 4. Cont.

Compound	RT	Molecular Formula	Theoretical <i>m/z</i>	Experimental <i>m/z</i> **	Relative Abundance (%) *				
					Inf	EPIInf	Mac	HSW Water	HSW Ethanol
Abscisic acid	10.04	C ₁₅ H ₂₀ O ₄	265.1434	265.1420	5.11	5.73	4.98	6.03	4.60
Quercitrin 2''-O-Gallate	10.25	C ₂₈ H ₂₄ O ₁₅	601.1187	601.1141	0.07	0.06	0.51	0.15	0.58
Afzelin	10.35	C ₂₁ H ₂₀ O ₁₀	433.1129	433.1088	0.55	0.56	8.10	0.60	0.82
Quercetin	10.57	C ₁₅ H ₁₀ O ₇	303.0499	303.0487	0.16	0.18	0.58	1.31	0.47
Linolenic acid	13.40	C ₁₈ H ₃₀ O ₂	279.2318	279.2293	0.29	0.30	1.02	2.77	0.46
Linoleic acid	14.66	C ₁₈ H ₃₂ O ₂	281.2475	281.2447	0.39	0.54	0.40	0.86	0.30
Flavonoids					29.59	31.89	56.67	28.05	52.76
Organic acids					61.37	57.51	35.60	59.25	40.00
Phenolic acids					1.04	1.47	0.64	1.66	1.41
Terpenoids					6.64	7.55	5.25	6.14	4.66
Fatty acids					1.36	1.58	1.84	4.90	1.16

Note: Inf—infusion; EPIInf—infusion followed by ethanol precipitation; Mac—maceration; HSW—high-shear homogenization-assisted extraction; RT—retention time (min). * Compound relative abundances were estimated based on the chromatographic peak areas and are presented as descriptive comparisons among the extracts. ** Mass errors and MS/MS fragments are provided in the Supplementary Material (Table S1).

Table 5. Identification of the constituents of *E. uniflora* extracts using ultra-high-performance liquid chromatography–tandem mass spectrometry (UHPLC–MS/MS) in negative mode.

Compound	RT	Molecular Formula	Theoretical <i>m/z</i>	Experimental <i>m/z</i> **	Relative Abundance (%) *				
					Inf	EPIInf	Mac	HSW Water	HSW Ethanol
Shikimic acid	1.59	C ₇ H ₁₀ O ₅	173.0450	173.0452	0.39	0.35	0.33	0.28	0.32
Quinic acid	1.69	C ₇ H ₁₂ O ₆	191.0561	191.0560	27.58	22.04	20.50	18.70	19.43
Saccharic acid	1.84	C ₆ H ₁₀ O ₈	209.0302	209.0305	7.01	5.29	4.34	6.29	4.62
Succinic acid	2.20	C ₄ H ₆ O ₄	117.0193	117.0193	2.84	2.84	3.07	2.70	2.74
Citric acid	2.54	C ₆ H ₈ O ₇	191.0193	191.0203	50.86	56.92	50.47	60.37	50.85
Gallic acid	2.70	C ₇ H ₆ O ₅	169.0140	169.0142	0.04	0.05	0.18	0.04	0.08
Caffeic acid hexoside	5.69	C ₁₅ H ₁₈ O ₉	341.0871	341.0878	0.47	0.61	0.61	0.61	0.62
Chlorogenic acid	6.47	C ₁₆ H ₁₈ O ₉	353.0867	353.0866	0.48	0.56	0.47	0.45	0.79
Myricetin O-pentoside (Isomer I)	7.66	C ₂₀ H ₁₈ O ₁₂	449.0714	449.0724	0.01	0.01	0.11	0.01	0.14
Myricitrin (Isomer I)	7.74	C ₂₁ H ₂₀ O ₁₂	463.0882	463.0876	0.03	0.05	0.37	0.03	0.45
Isorhamnetin 3-galactoside	7.99	C ₂₂ H ₂₂ O ₁₂	477.1038	477.1026	0.12	0.14	0.40	0.12	0.35
Coumaric acid	8.01	C ₉ H ₈ O ₃	163.0400	163.0400	0.14	0.13	0.14	0.21	0.11

Table 5. Cont.

Compound	RT	Molecular Formula	Theoretical <i>m/z</i>	Experimental <i>m/z</i> **	Relative Abundance (%) *				
					Inf	EPInf	Mac	HSH Water	HSH Ethanol
Myricetin O-galactoside	8.11	C ₂₁ H ₂₀ O ₁₃	479.0831	479.0828	0.05	0.05	0.47	0.01	0.53
Myricetin O-pentoside (Isomer II)	8.61	C ₂₀ H ₁₈ O ₁₂	449.0714	449.0723	0.05	0.05	0.43	0.01	0.45
Myricitrin (Isomer II)	8.73	C ₂₁ H ₂₀ O ₁₂	463.0882	463.0870	0.54	0.67	1.94	0.27	1.97
Rutin	8.97	C ₂₇ H ₃₀ O ₁₆	609.1461	609.1463	0.20	0.25	0.31	0.23	0.31
Isoquercitrin	9.01	C ₂₁ H ₂₀ O ₁₂	463.0882	463.0870	1.46	1.68	3.33	1.37	3.43
Quercetin O-pentoside	9.49	C ₂₀ H ₁₈ O ₁₁	433.0765	433.0770	1.04	1.15	3.14	0.85	3.18
Kaempferol-3-glucoside	9.60	C ₂₁ H ₂₀ O ₁₁	447.0932	447.0929	0.12	0.13	0.21	0.11	0.20
Quercitrin	9.73	C ₂₁ H ₂₀ O ₁₁	447.0932	447.0924	4.37	4.61	6.44	4.67	6.59
Abscisic acid	10.06	C ₁₅ H ₂₀ O ₄	263.1285	263.1289	1.96	2.10	2.09	2.13	2.16
Kaempferol	10.11	C ₂₀ H ₁₈ O ₁₀	417.0827	417.0834	0.07	0.08	0.20	0.06	0.21
alpha-L-arabinopyranoside	10.37	C ₂₁ H ₂₀ O ₁₀	431.0983	431.0983	0.16	0.18	0.34	0.20	0.36
Quercetin	10.59	C ₁₅ H ₁₀ O ₇	301.0353	301.0356	0.02	0.02	0.11	0.27	0.11
Flavonoids					8.24	9.09	17.80	8.23	18.29
Organic acids					88.68	87.45	78.72	88.33	77.96
Phenolic acids					1.13	1.35	1.40	1.31	1.60
Terpenoids					1.96	2.10	2.09	2.13	2.16

Note: Inf—infusion; EPInf—infusion followed by ethanol precipitation; Mac—maceration; HSH—high-shear homogenization-assisted extraction; RT—retention time (min). * Compound relative abundances were estimated based on the chromatographic peak areas and are presented as descriptive comparisons among the extracts. ** Mass errors and MS/MS fragments are provided in the Supplementary Material (Table S1).

4. Discussion

Extraction yield varied significantly among the evaluated procedures, demonstrating that the overall recovery of constituents from *E. uniflora* fruits depends on the combined influence of extraction technique and solvent. Among the evaluated treatments, HSH using 70% ethanol and ethanolic maceration produced the highest extraction yields. Although no significant differences were observed between these procedures, they differ substantially in terms of processing time and operational characteristics. While ethanolic maceration required seven days of solvent–matrix contact, HSH achieved a comparable yield after only 10 min, highlighting the potential of mechanically assisted extraction to intensify the extraction process.

The high extraction efficiency achieved by HSH is likely associated with the intense mechanical forces generated by the rotor–stator system. High shear promotes disruption of plant tissues, particle size reduction, and enlargement of the contact surface between the plant matrix and the extraction solvent, thereby reducing mass-transfer limitations and facilitating the release of intracellular constituents. Similar mechanisms have been associated with enhanced extraction efficiency in different plant matrices subjected to HSH [8–10].

However, mechanical disruption alone was insufficient to ensure high extraction efficiency. Under identical extraction conditions of processing time, temperature, homogenization speed and solid-to-solvent ratio, HSH performed with water produced an extraction yield approximately 3.2-fold lower than that obtained using 70% ethanol. This direct comparison demonstrates that solvent composition exerted a decisive influence on extraction performance. Water preferentially solubilizes highly polar constituents, whereas hydroethanolic mixtures broaden the polarity range of extracted compounds, enabling the simultaneous recovery of hydrophilic constituents and metabolites of intermediate polarity, including several phenolic compounds and flavonoids [2,7,10].

The superior performance of the hydroethanolic system may also be explained by the complementary roles of water and ethanol during extraction. Water promotes hydration and swelling of plant tissues, facilitating solvent penetration into the cellular matrix, whereas ethanol exhibits greater affinity for numerous phenolic metabolites. Consequently, the 70% hydroethanolic mixture combines efficient tissue permeation with enhanced solubilization of released bioactive compounds [7,10]. Nevertheless, because the remaining extraction procedures were not evaluated under a fully crossed experimental design regarding extraction technique and solvent, the isolated effect of solvent should be interpreted with greater confidence only when comparing the two HSH extracts.

The high extraction yield obtained by ethanolic maceration can likewise be attributed to prolonged solvent–matrix contact. The seven-day extraction period probably favored gradual diffusion of soluble constituents until concentration equilibrium was reached. Nevertheless, achieving a yield comparable to that obtained by HSH required a processing time approximately 1000-fold longer. Although the present study did not evaluate energy consumption or process economics, this marked operational difference indicates a potential technological advantage of HSH for applications requiring process intensification, shorter extraction times and increased productivity [7].

The benefits of process intensification through HSH have also been reported for other botanical matrices. Teixeira et al. [15] optimized HSH conditions for *Malpighia emarginata* fruits and achieved efficient recovery of bioactive compounds after only 5 min by adjusting the solid-to-solvent ratio, homogenization speed and temperature. Similarly, Guerboub et al. [10] demonstrated that HSH conditions significantly influenced both polyphenol recovery and antioxidant activity in olive pomace extracts. Together, these studies reinforce that short-duration mechanical extraction techniques can achieve extrac-

tion efficiencies comparable to those obtained by conventional long-term procedures, although their effectiveness remains dependent on the characteristics of the plant matrix and extraction conditions.

Among the aqueous procedures, infusion produced an intermediate extraction yield, indicating that the combination of elevated initial temperature and prolonged contact with water enabled the solubilization of a considerable fraction of *E. uniflora* constituents. Heating generally enhances diffusion processes, reduces solvent viscosity and improves solvent penetration into plant tissues [13]. In contrast, EPIInf resulted in a lower extraction yield. However, this reduction should not be interpreted solely as lower extraction efficiency because the addition of 95% ethanol was intended to selectively precipitate proteins, polysaccharides and other high-molecular-weight constituents.

Therefore, the lower gravimetric yield reflects not only reduced recovered mass but also an intentional modification of extract composition, potentially increasing extract selectivity. This observation illustrates that extraction yield alone does not necessarily reflect extract quality or biological functionality [13]. Accordingly, extraction procedures should be evaluated by integrating extraction yield with biological activities and chemical composition rather than considering recovered mass as the sole criterion for selecting the most suitable extraction method.

Comparison with previous studies involving *E. uniflora* fruits (Table 6) further supports the importance of simultaneously considering solvent composition and extraction technology. Migueis et al. [5] employed a methanol–water–formic acid mixture combined with two prolonged ultrasound-assisted extraction steps, obtaining extracts rich in anthocyanins and derivatives of myricetin and quercetin. Although highly efficient for analytical purposes, this procedure requires methanol and considerably longer processing times, limiting its direct applicability to nutraceutical ingredient production.

Table 6. Comparison of the present study with previous studies on extraction procedures, chemical composition, and biological activities of *E. uniflora* fruits.

Study	Plant Material	Extraction Procedure	Assessments and Main Findings	Relationship to the Present Study
Present study	Ripe red-pulp pitanga fruits, seedless, lyophilized and powdered.	Infusion; EPIInf; maceration with 70% ethanol; HSH with water or 70% ethanol.	Extraction yield, FRAP, ABTS ^{•+} , DPPH, MIC, and UHPLC–MS/MS. Ethanolic HSH showed the best overall performance; maceration exhibited the highest ABTS ^{•+} response.	Compares five extraction procedures, integrating extraction performance, biological activities, and chemical profiling.
Migueis et al. [5]	Purple-pulp fruits, lyophilized and collected from different regions of Uruguay.	Methanol:water:formic acid (70:29:1); vortexing, sonication for 60 min, overnight standing at 4 °C, followed by a second 60 min sonication.	HPLC-DAD-ESI/MS ⁿ , phenolic compounds, DPPH, and ORAC. Two anthocyanins and 13 flavonols were reported, with emphasis on myricetin derivatives.	Corroborates the chemical classes observed in the present study but employs methanol and a prolonged analytical procedure.

Table 6. Cont.

Study	Plant Material	Extraction Procedure	Assessments and Main Findings	Relationship to the Present Study
Oliveira et al. [21]	Whole red fruits, frozen.	Sonication for 30 min at 25 °C using ethanol:water (70:30), acidified to pH 1.0.	Phenolic compounds, flavonoids, anthocyanins, LC/PDA/MS/MS, and an in vivo study. The standardized extract exhibited antioxidant and metabolic effects in the evaluated model.	The closest comparison regarding the hydroethanolic solvent and mechanical processing, although a different biological endpoint was investigated.
Rashmi and Negi [22]	Fruit pulp and seeds at different maturation stages.	Ethanol extraction of different fruit parts and maturation stages.	DPPH, FRAP, UPLC–MS, UPLC-ESI-TOF-MS, and anthelmintic activity. Sixteen phenolic compounds were detected.	Shows that plant part and maturation stage affect chemical composition and bioactivity; numerical data are provided for context only.
Gonçalves et al. [6]	Ripe fruits of orange- and purple-pulp varieties, lyophilized and powdered.	μ-QuEChERS using acetonitrile:ethyl acetate (1:1), acidified; vortexing, 5 min sonication, and dSPE cleanup.	Phenolic compounds, flavonoids, anthocyanins, DPPH, ABTS ^{•+} , and UHPLC-PDA. Gallic acid, quercetin, and other phenolic compounds were quantified according to fruit variety.	Employs an analytical procedure that does not directly represent ingredient production.
Rodrigues et al. [23]	Pitanga fruit pulp.	Preparation of a phenolic extract from the pulp.	Chemical composition, antioxidant activity, antimicrobial activity, and quorum-sensing inhibition. The extract inhibited bacteria and reduced violacein production.	Corroborates the antibacterial activity of the phenolic fraction, although methods, concentrations, and biological endpoints differed from the present study.
Bagetti et al. [4]	Red-, orange-red-, and purple-pulp fruits.	Fruit characterization and preparation of extracts for antioxidant evaluation.	Assessed physicochemical composition, phenolic compounds, anthocyanins, and antioxidant activity; the purple variety showed the highest values.	Highlights the effects of genotype and fruit color, which limit direct comparisons among studies.

Note: EPInf—infusion followed by ethanol precipitation; HSH—high-shear homogenization-assisted extraction. Comparisons are predominantly methodological, chemical, and qualitative. Quantitative results should not be directly compared because of differences in fruit variety, plant part, maturation stage, sample preparation, solvent, extraction procedure, analytical assay, and unit of expression.

In contrast, Oliveira et al. [21] employed a 70:30 ethanol–water mixture associated with 30 min of sonication and obtained anthocyanin-rich extracts exhibiting antioxidant and metabolic effects in an animal model. These findings reinforce the suitability of hydroethanolic solvents for recovering functional compounds from pitanga fruits. In the present study, combining 70% ethanol with HSH reduced extraction time to only 10 min while maintaining high extraction yield and producing extracts with both antioxidant and antibacterial activities, supporting this approach as a rapid and efficient alternative for obtaining bioactive extracts with potential nutraceutical applications.

The extraction procedure influenced the antioxidant capacity of *E. uniflora* fruit extracts, although the magnitude of this effect depended on the analytical method employed. HSH using 70% ethanol showed the highest antioxidant activity in the FRAP and DPPH assays, whereas ethanolic maceration exhibited the highest activity in the ABTS^{•+} assay. These differences were expected because each assay is based on distinct reaction mechanisms and therefore responds differently to the chemical composition of the extracts. While FRAP measures the reducing capacity of the sample through electron transfer, DPPH and ABTS^{•+} estimate radical-scavenging activity under different reaction conditions [11]. Consequently, discrepancies among the assays should not be interpreted as analytical inconsistencies but rather as evidence that different extraction procedures favored the recovery of compounds acting through distinct antioxidant mechanisms.

The superior performance of the HSH ethanolic extract in the FRAP assay suggests that this extraction procedure favored the recovery of constituents with high reducing power. Because the FRAP assay measures the ability of antioxidants to reduce ferric ions (Fe³⁺) to ferrous ions (Fe²⁺), it is particularly sensitive to compounds capable of efficient electron donation [11]. The combination of intense mechanical disruption and the broad extraction capacity of the hydroethanolic solvent probably enhanced the release of these compounds by reducing mass-transfer limitations and increasing solvent accessibility to intracellular constituents [8–10].

A similar trend was observed in the DPPH assay, in which HSH using 70% ethanol produced substantially higher radical-scavenging activity than all other extraction procedures. In contrast, the aqueous infusion showed comparatively low activity, whereas EPInf, ethanolic maceration and aqueous HSH produced values below the quantification limit. Importantly, these findings should not be interpreted as the absence of antioxidant compounds in those extracts, as the same extracts exhibited measurable activity in the FRAP and ABTS^{•+} assays. Therefore, the DPPH results should be interpreted together with complementary antioxidant methods, since the response of each assay depends on the chemical characteristics and reactivity of the compounds present in the extracts [11].

Unlike the FRAP and DPPH assays, the ABTS^{•+} assay identified ethanolic maceration as the most effective extraction procedure, reaching 170.59 $\mu\text{mol TE g}^{-1}$. The markedly different response of this extract between the DPPH and ABTS^{•+} assays may be related to differences in the reaction characteristics and antioxidant sensitivity of these methods. ABTS^{•+} can react with a broad range of hydrophilic and lipophilic antioxidant compounds, whereas DPPH may exhibit different reactivity depending on the chemical characteristics and accessibility of the antioxidants present [11]. Thus, the absence of detectable DPPH activity in the ethanolic maceration extract, despite its high ABTS^{•+} activity, likely reflects a differential response to the assay conditions rather than the absence of antioxidant constituents. The higher ABTS^{•+} response may also indicate that prolonged contact between the plant matrix and the hydroethanolic solvent favored the recovery of constituents particularly reactive toward this radical. These findings further reinforce that no single antioxidant assay is sufficient to comprehensively characterize the antioxidant potential of plant extracts.

The precipitation step performed after aqueous infusion also influenced antioxidant capacity. Compared with the original infusion, EPI_{Inf} reduced both FRAP and ABTS^{•+} responses. Since this procedure was specifically designed to precipitate proteins, polysaccharides and other high-molecular-weight constituents [13], the reduction in antioxidant activity likely reflects the partial removal of compounds contributing to the overall reducing capacity of the extract. Accordingly, the lower antioxidant response should not be interpreted solely as reduced extraction efficiency but rather as a consequence of the selective modification of extract composition.

Taken together, the antioxidant results demonstrate that extraction conditions affected not only the amount of recovered material but also the functional characteristics of the extracts. HSH using 70% ethanol produced the most consistent antioxidant profile, showing superior performance in two of the three assays evaluated, whereas ethanolic maceration preferentially recovered compounds exhibiting greater activity in the ABTS^{•+} assay. These findings indicate that there is no universally superior extraction procedure for all antioxidant responses. Instead, the most appropriate extraction strategy depends on the intended application and on the antioxidant mechanism considered most relevant.

The exploratory UHPLC–MS/MS analysis provides chemical support for the differences observed among the extraction procedures. Although the present study did not include absolute quantification of individual metabolites, comparison of the chromatographic peak areas allowed a descriptive evaluation of the relative abundance of compounds detected in the extracts. Consequently, the chemical profile should be interpreted as complementary evidence that helps explain the biological responses rather than as a quantitative demonstration of compound concentration.

Among the evaluated extracts, HSH using 70% ethanol exhibited larger relative peak areas for several flavonoids, including derivatives annotated as myricetin, quercetin, isoquercitrin, glycosylated kaempferol and afzelin (Tables 4 and 5). These metabolites have been consistently reported in *E. uniflora* fruits [5,6] and are recognized for structural characteristics that enable electron or hydrogen donation, particularly the presence of hydroxyl groups associated with conjugated aromatic systems [11]. Although these structural features provide a plausible explanation for the superior antioxidant performance of the HSH ethanolic extract, the chromatographic data alone do not establish a direct causal relationship between individual metabolites and the measured biological activities [24].

Likewise, the chemical profile of the ethanolic maceration extract may help explain its superior performance in the ABTS^{•+} assay. Compared with the remaining extraction procedures, this extract exhibited relatively higher peak areas for compounds such as isorhamnetin-3-galactoside, afzelin and succinic acid. Because ABTS^{•+} is responsive to antioxidants with a broad range of physicochemical properties [11], differences in the composition of these extracts probably contributed to the distinct responses observed among antioxidant assays. Therefore, variations in antioxidant activity are more likely associated with differences in the overall phytochemical composition recovered by each extraction procedure than with the abundance of a single metabolite [25].

Comparison with previous phytochemical studies further supports the identification of compounds consistent with those previously reported in *E. uniflora*. Derivatives of myricetin and quercetin have previously been described as major constituents of *E. uniflora* fruits, while gallic acid, quercetin, kaempferol and related phenolic compounds have also been reported in fruits of different color varieties [4–6,21,22]. Nevertheless, direct comparison among studies should be performed with caution because metabolite profiles are influenced by several factors, including genotype, fruit maturation stage, geographical origin, sample preparation, extraction solvent and extraction technique. For this reason, agreement regarding the classes of metabolites identified is more informative than direct

comparison of chromatographic responses or antioxidant values obtained under different experimental conditions [26,27].

The limitations of comparing chromatographic peak areas should also be recognized. Since the analysis was performed without external analytical standards, peak areas do not represent absolute concentration and cannot be used to quantify differences among chemically distinct metabolites. Therefore, the present results should be interpreted as descriptive evidence indicating that extraction conditions altered the relative chemical composition of the extracts. Overall, the UHPLC–MS/MS analysis indicates that the extraction procedure influenced not only extraction yield but also the phytochemical profile. The predominance of flavonoids and other phenolic constituents in the HSH ethanolic extract is chemically consistent with its superior antioxidant performance and provides a plausible basis for the antibacterial activity discussed below. However, because the extracts constitute complex mixtures, the observed biological effects should be interpreted as the result of the combined action of multiple constituents rather than being attributed to any single annotated compound [24].

In addition to affecting extraction yield and antioxidant capacity, extraction conditions markedly influenced the antibacterial potential of *E. uniflora* fruit extracts. The direct comparison between HSH performed with water and 70% ethanol clearly demonstrates the decisive role of solvent selection. Under identical extraction conditions, including extraction time, homogenization speed, temperature and solid-to-solvent ratio, the ethanolic extract inhibited five of the six tested microorganisms, whereas the aqueous extract showed no detectable activity at the highest concentration evaluated. This finding indicates that solvent composition played a central role in determining the recovery of antibacterial constituents from pitanga fruits.

The superior antibacterial activity observed for the HSH ethanolic extract is chemically consistent with its phytochemical profile. UHPLC–MS/MS analysis revealed a greater relative abundance of flavonoids and other phenolic compounds compared with the aqueous extract, suggesting that hydroethanolic extraction favored the recovery of metabolites previously associated with antibacterial activity [2,28]. Nevertheless, because the present study involved exploratory metabolite annotation and comparative analysis of chromatographic peak areas, these observations should be interpreted as evidence of chemical plausibility rather than proof of a direct relationship between individual compounds and antimicrobial activity.

Among the annotated metabolites, flavonoids deserve particular attention because they have been widely associated with antibacterial mechanisms. Studies employing isolated quercetin provide support for this interpretation. Wang et al. [29] demonstrated antibacterial activity of quercetin against both Gram-positive and Gram-negative bacteria and reported structural alterations in *E. coli* and *S. aureus*. Similarly, Mu et al. [30] observed that quercetin reduced extracellular polymeric substance production and inhibited biofilm formation by *S. epidermidis*. Although these findings reinforce the biological relevance of flavonoids, they cannot be directly extrapolated to the present extracts. The biological activity observed here most likely results from the combined action of multiple constituents acting through additive or synergistic mechanisms rather than from the effect of any single annotated metabolite [31].

Accordingly, the principal contribution of the antimicrobial assays is not to identify the active compound responsible for bacterial inhibition, but rather to demonstrate that extraction conditions modulated the recovery of the antibacterial fraction present in *E. uniflora* fruits. From this perspective, HSH using 70% ethanol proved to be the most efficient procedure among those evaluated, producing an extract with consistently superior

antibacterial activity while simultaneously exhibiting favorable antioxidant performance and a phytochemical profile enriched in phenolic constituents.

No extract inhibited the growth of *C. albicans* under the experimental conditions evaluated. This result agrees with previous observations reported for *E. uniflora* leaves, in which antifungal activity against *C. albicans* was likewise not detected [31]. However, these findings should not be interpreted as evidence that *E. uniflora* fruits are devoid of antifungal potential. Differences in extraction solvent, fractionation strategy, extract concentration or experimental methodology may influence antifungal activity. Therefore, within the scope of the present study, the antimicrobial potential of pitanga fruit extracts should be considered predominantly antibacterial.

From a nutraceutical perspective, antibacterial activity may represent an additional functional property that complements the antioxidant potential of the extracts. Nevertheless, these findings should not be interpreted as supporting therapeutic claims. Before any practical application can be proposed, further investigations will be required to evaluate cytotoxicity, safety, stability, performance in food matrices or formulated products, and possible effects on beneficial microorganisms [32]. Such studies will be essential to establish whether the antibacterial properties observed in vitro can be translated into safe and effective applications.

From a technological and nutraceutical perspective, HSH using 70% ethanol emerged as the most promising extraction strategy among the evaluated procedures, combining high extraction yield, short processing time, antioxidant capacity, and antibacterial activity. The use of an ethanol–water mixture is particularly relevant for nutraceutical applications because ethanol is generally recognized as safe (GRAS) [13], supporting its suitability for the development of food-oriented bioactive extracts. The exploratory UHPLC–MS/MS profile further supports the nutraceutical potential of the selected extract, with citric acid, quinic acid, and quercitrin showing prominent chromatographic peak areas among the annotated compounds. Nevertheless, the transition from a promising extract to a standardized nutraceutical ingredient requires further process optimization and scale-up, as well as validated quantitative methods and appropriate chemical markers to ensure batch-to-batch reproducibility.

Despite these promising findings, some limitations should be acknowledged before considering the extract for nutraceutical development. Toxicological safety was not evaluated; therefore, the observed in vitro activities cannot be directly extrapolated to the safety of the extracts for human consumption. In addition, organoleptic characteristics and consumer acceptability were not assessed, and the stability of the major constituents during storage was not investigated. Future studies should address these aspects, together with bioaccessibility, bioavailability, and in vivo efficacy, to establish the safety, stability, reproducibility, and biological effectiveness required for the further development of pitanga fruit extracts as nutraceutical ingredients.

5. Conclusions

The extraction procedures significantly influenced the extraction yield, antioxidant capacity, antibacterial activity, and chemical profile of *E. uniflora* fruit extracts. HSH with 70% ethanol exhibited the most promising overall performance, combining a high extraction yield obtained within only 10 min, greater reducing capacity in the FRAP assay, the strongest response in the DPPH assay, and lower minimum inhibitory concentrations against several bacterial species.

The exploratory UHPLC–MS/MS profiling revealed a diverse chemical composition, with annotated signals corresponding to organic acids, phenolic acids, free and glycosylated flavonoids, and other plant metabolites. Overall, these findings indicate that HSH with

70% ethanol is a promising strategy for the rapid production of a pitanga fruit extract with nutraceutical potential. However, further development will require chemical standardization across batches, safety and stability assessments, evaluation of efficacy in biological models, and process validation at larger scale.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/nutraceuticals6040066/s1>; Table S1: UHPLC–MS/MS annotation of metabolites detected in *Eugenia uniflora* L. fruit extracts in positive- and negative-ionization modes, including mass errors and diagnostic MS/MS fragments.

Author Contributions: Conceptualization, L.G.P. and E.L.B.L.; methodology, L.G.P., M.D., L.A.E., T.A.d.A., N.N.S., P.D.M., E.J.P., E.D.P., O.A., E.L.B.L. and J.H.; software, P.D.M., E.J.P. and O.A.; validation, M.D., L.A.E., T.A.d.A., N.N.S., P.D.M. and J.H.; formal analysis, M.D., L.A.E., T.A.d.A., N.N.S. and P.D.M.; investigation, L.G.P., P.D.M., E.J.P., O.A., E.L.B.L. and J.H.; resources, L.G.P., M.D., L.A.E., T.A.d.A., N.N.S. and P.D.M.; data curation, E.J.P., E.D.P., O.A., E.L.B.L. and J.H.; writing—original draft preparation, L.G.P., E.D.P. and E.L.B.L.; writing—review and editing, M.D., L.A.E., T.A.d.A., N.N.S., P.D.M., O.A. and J.H.; visualization, J.H.; supervision, E.L.B.L.; project administration, L.G.P. and E.L.B.L.; funding acquisition, E.J.P., O.A. and E.L.B.L. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The datasets generated and/or analyzed during the current study are available within the manuscript. Additional information and supporting data are available from the corresponding author upon request.

Acknowledgments: The authors acknowledge Universidade Paranaense (UNIPAR), CAPES, Fundação Cândido Garcia and CNPq for supporting the research. L.G.P., M.D., and L.A.E. thank PROSUP/CAPES for the scholarship support. O.A., E.L.B.L., and J.H. acknowledge a research fellowship from the National Council for Scientific and Technological Development (CNPq).

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

ABTS	2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)
DPPH	2,2-Diphenyl-1-picrylhydrazyl
EPInf	Infusion followed by ethanol precipitation
FRAP	Ferric reducing antioxidant power
HSH	High-shear homogenization-assisted extraction
Inf	Infusion
Mac	Maceration
MIC	Minimum inhibitory concentration
RT	Retention time
UHPLC–MS/MS	Ultra-high-performance liquid chromatography–tandem mass spectrometry
TE	Trolox equivalents

References

1. Najmi, A.; Javed, S.A.; Al Bratty, M.; Alhazmi, H.A. Modern Approaches in the Discovery and Development of Plant-Based Natural Products and Their Analogues as Potential Therapeutic Agents. *Molecules* **2022**, *27*, 349. [CrossRef]
2. Chemat, F.; Vian, M.A.; Cravotto, G. Green Extraction of Natural Products: Concept and Principles. *Int. J. Mol. Sci.* **2012**, *13*, 8615–8627. [CrossRef]

3. Fidelis, E.M.; Savall, A.S.P.; Pereira, F.O.; Quines, C.B.; Ávila, D.S.; Pinton, S. Pitanga (*Eugenia uniflora* L.) as a Source of Bioactive Compounds for Health Benefits: A Review. *Arab. J. Chem.* **2022**, *15*, 103691. [CrossRef]
4. Bagetti, M.; Facco, E.M.P.; Piccolo, J.; Hirsch, G.E.; Rodriguez-Amaya, D.B.; Kobori, C.N.; Emanuelli, T. Physicochemical Characterization and Antioxidant Capacity of Pitanga Fruits (*Eugenia uniflora* L.). *Food Sci. Technol.* **2011**, *31*, 147–154. [CrossRef]
5. Migueis, I.; Baenas, N.; Gironés-Vilaplana, A.; Cesio, M.V.; Heinzen, H.; Moreno, D.A. Phenolic Profiling and Antioxidant Capacity of *Eugenia uniflora* L. (Pitanga) Samples Collected in Different Uruguayan Locations. *Foods* **2018**, *7*, 67. [CrossRef]
6. Gonçalves, J.; Hontman, N.; Perestrelo, R.; Câmara, J.S. A Comparative Study of the Biological Properties of *Eugenia uniflora* L. Fruits and Leaves Related to the Prevention of Cardiovascular Diseases. *Life* **2025**, *15*, 147. [CrossRef]
7. Martínez-Correa, H.A.; Magalhães, P.M.; Queiroga, C.L.; Peixoto, C.A.; Oliveira, A.L.; Cabral, F.A. Extracts from Pitanga (*Eugenia uniflora* L.) Leaves: Influence of Extraction Process on Antioxidant Properties and Yield of Phenolic Compounds. *J. Supercrit. Fluids* **2011**, *55*, 998–1006. [CrossRef]
8. Vázquez-Vázquez, J.; Barajas-Salazar, M.; Casas-Godoy, L.; Alcázar-Valle, M.; Arellano-García, L.; Barrera-Martínez, I. Enhanced Extraction of Antioxidant Phenolics: Quercetin, Kaempferol, Gallic Acid, Syringic Acid, and Epicatechin from Fresh Berries and Their Waste Using Ultra-Turrax and Ultrasonication. *Appl. Food Res.* **2024**, *4*, 100539. [CrossRef]
9. Kamble, M.G.; Singh, A.; Prabhakar, P.K.; Meghwal, M.; Singh, S.V.; Chinchkar, A.V. Effect of High Shear Homogenization on Quality Characteristics of Bael Fruit Pulp. *Food Qual. Saf.* **2022**, *6*, fyac011. [CrossRef]
10. Guerboub, L.; Soufi-Maddi, O.; Ouldsaadi, L.; Bachir-Bey, M.; Medouni-Haroune, L.; Madani, K.; Boulekbache-Makhlouf, L. Ultra-Turrax Homogenization-Assisted Extraction of Bioactive Compounds from Olive Pomace Using Response Surface Methodology. *Food Anal. Methods* **2025**, *18*, 2198–2207. [CrossRef]
11. Huang, D.; Ou, B.; Prior, R.L. The Chemistry behind Antioxidant Capacity Assays. *J. Agric. Food Chem.* **2005**, *53*, 1841–1856. [CrossRef]
12. Wenas, D.M.; Elya, B.; Sutriyo, S.; Setiawan, H.; Othman, R.; Nur, S.; Triadisti, N.; Yunita, F.; Setyaningsih, E.P. In Vitro and In Silico Evaluation of the Anti-Aging Potential of *Eugenia uniflora* UAE Extracts. *Molecules* **2025**, *30*, 3168. [CrossRef]
13. Pinc, M.M.; Dalmagro, M.; da Cruz Alves Pereira, E.; Donadel, G.; Thomaz, R.T.; da Silva, C.; Macruz, P.D.; Jacomassi, E.; Gasparotto Junior, A.; Hoscheid, J.; et al. Extraction Methods, Chemical Characterization, and In Vitro Biological Activities of *Plinia cauliflora* (Mart.) Kausel Peels. *Pharmaceuticals* **2023**, *16*, 1173. [CrossRef]
14. Tominc, G.C.; Dalmagro, M.; Scheneider, N.K.; Maltauro, A.P.; Zanella, G.C.; Seren, A.P.; Zardeto, G.; Hoscheid, J. Otimização de método extrativo para raízes de *Arctium lappa* L. *Arq. Ciênc. Saúde. UNIPAR* **2022**, *26*, 1019–1032. Available online: <https://revistas.unipar.br/index.php/saude/article/view/8975> (accessed on 13 August 2026).
15. Teixeira, G.R.; Dalmagro, M.; Macruz, P.D.; Pilau, E.J.; Lourenço, E.L.B.; Hoscheid, J. Extractive Optimisation of Bioactive Compounds from *Malpighia emarginata* DC. through Box–Behnken Experimental Design. *Nat. Prod. Res.* **2026**, 1–6. [CrossRef]
16. Benzie, I.F.F.; Strain, J.J. The Ferric Reducing Ability of Plasma (FRAP) as a Measure of “Antioxidant Power”: The FRAP Assay. *Anal. Biochem.* **1996**, *239*, 70–76. [CrossRef]
17. Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant Activity Applying an Improved ABTS Radical Cation Decolorization Assay. *Free Radic. Biol. Med.* **1999**, *26*, 1231–1237. [CrossRef]
18. Brand-Williams, W.; Cuvelier, M.E.; Berset, C. Use of a Free Radical Method to Evaluate Antioxidant Activity. *LWT-Food Sci. Technol.* **1995**, *28*, 25–30. [CrossRef]
19. Clinical and Laboratory Standards Institute. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*; Approved Standard—Ninth Edition; CLSI Document M07-A9; CLSI: Wayne, PA, USA, 2012.
20. Sumner, L.W.; Amberg, A.; Barrett, D.; Beale, M.H.; Beger, R.; Daykin, C.A.; Fan, T.W.-M.; Fiehn, O.; Goodacre, R.; Griffin, J.L.; et al. Proposed minimum reporting standards for chemical analysis. *Metabolomics* **2007**, *3*, 211–221. [CrossRef]
21. Oliveira, P.S.; Chaves, V.C.; Bona, N.P.; Soares, M.S.P.; Cardoso, J.S.; Vasconcellos, F.A.; Tavares, R.G.; Vizzotto, M.; Silva, L.M.C.; Grecco, F.B.; et al. *Eugenia uniflora* Fruit (Red Type) Standardized Extract: A Potential Pharmacological Tool to Diet-Induced Metabolic Syndrome Damage Management. *Biomed. Pharmacother.* **2017**, *92*, 935–941. [CrossRef]
22. Rashmi, H.B.; Negi, P.S. Phytochemical Constituents and Anthelmintic Potential of Surinam Cherry (*Eugenia uniflora* L.) at Different Fruit Developmental Stages. *S. Afr. J. Bot.* **2022**, *145*, 512–521. [CrossRef]
23. Rodrigues, A.C.; Zola, F.G.; Oliveira, B.D.A.; Sacramento, N.T.B.; Silva, E.R.; Bertoldi, M.C.; Taylor, J.G.; Pinto, U.M. Quorum Quenching and Microbial Control through Phenolic Extract of *Eugenia uniflora* Fruits. *J. Food Sci.* **2016**, *81*, M2538–M2544. [CrossRef]
24. Huynh, K.N.N.; Nguyen, N.V.T.; Ngan, D.T. Natural Phenolic Compounds: A Review of Extraction and Analysis. In *Exploring Natural Phenolic Compounds—Recent Progress and Practical Applications*; Gouvinhas, I., Barros, A.N., Eds.; IntechOpen: London, UK, 2024. [CrossRef]
25. Villanueva-Bermejo, D.; Siles-Sánchez, M.N.; Hernández, D.M.; García-Risco, M.R.; Jaime, L.; Santoyo, S.; Fornari, T. Theoretical Framework to Evaluate Antioxidant Synergistic Effects from the Coextraction of Marjoram, Rosemary and Parsley. *Food Chem.* **2024**, *437*, 137919. [CrossRef]

26. Nurzyńska-Wierdak, R. Phenolic Compounds from New Natural Sources—Plant Genotype and Ontogenetic Variation. *Molecules* **2023**, *28*, 1731. [[CrossRef](#)]
27. Lisjak, M.; Špoljarević, M.; Ravlić, J.; Lončarić, Z.; Galić, L. Phenolic Compounds and Antioxidant Activity: Analytical Methods and Current Knowledge—A Review. *Methods Protoc.* **2026**, *9*, 60. [[CrossRef](#)]
28. Dembińska, K.; Shinde, A.H.; Pejchalová, M.; Richert, A.; Swiontek Brzezinska, M. The Application of Natural Phenolic Substances as Antimicrobial Agents in Agriculture and Food Industry. *Foods* **2025**, *14*, 1893. [[CrossRef](#)]
29. Wang, S.; Yao, J.; Zhou, B.; Yang, J.; Chaudry, M.T.; Wang, M.; Xiao, F.; Li, Y.; Yin, W. Bacteriostatic Effect of Quercetin as an Antibiotic Alternative In Vivo and Its Antibacterial Mechanism In Vitro. *J. Food Prot.* **2018**, *81*, 68–78. [[CrossRef](#)]
30. Mu, Y.; Zeng, H.; Chen, W. Quercetin Inhibits Biofilm Formation by Decreasing the Production of EPS and Altering the Composition of EPS in *Staphylococcus epidermidis*. *Front. Microbiol.* **2021**, *12*, 631058. [[CrossRef](#)]
31. Dalmagro, M.; Pinc, M.M.; Donadel, G.; Tominc, G.C.; Jacomassi, E.; Lourenço, E.L.B.; Gasparotto Junior, A.; Boscarato, A.G.; Belettini, S.T.; Alberton, O.; et al. Bioprospecting a Film-Forming System Loaded with *Eugenia uniflora* L. and *Tropaeolum majus* L. Leaf Extracts for Topical Application in Treating Skin Lesions. *Pharmaceuticals* **2023**, *16*, 1068. [[CrossRef](#)]
32. Santini, A.; Cammarata, S.M.; Capone, G.; Ianaro, A.; Tenore, G.C.; Pani, L.; Novellino, E. Nutraceuticals: Opening the Debate for a Regulatory Framework. *Br. J. Clin. Pharmacol.* **2018**, *84*, 659–672. [[CrossRef](#)]

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.