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Phytochemical Extract from *Syzygium cumini* Leaf: Maximization of Compound Extraction, Chemical Characterization, Antidiabetic and Antibacterial Activity, and Cell Viability

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Abstract: This work aimed to obtain a phytochemical extract from jambolan leaf using a hydroethanolic solvent and ultrasound-assisted extraction. For this purpose, an experimental design was applied to analyze the effect of process variables related to temperature (30–60 °C), time (10–30 min), and solvent to leaf ratio (5–15 mL g^{-1}), on the extraction mass yield (EMY) and on the yield of phenolic compounds (PCY). The effect of extractor solvent, AE (absolute ethanol), 75E (75% $v{\cdot}v^{-1}$ ethanol) and 50E (50% $v{\cdot}v^{-1}$), on the chemical characterization of the extracts, antidiabetic and antimicrobial activity, and cell viability, were also evaluated. The application of the highest values of process variables resulted in obtaining the maximum of the response variables (EMY = 9.94 wt% and PCY = 13.01 mg GAE g^{-1} leaf). A higher content of phenolic compounds and flavonoids was obtained with 50E, which is mainly composed of sinapic, vanillic, *trans*-caffeic, and quinic acids, which were responsible for the greatest antioxidant potential, antibacterial activity (against *Staphylococcus aureus* and *Pseudomonas aeruginosa*), and inhibition of α-amylase. On the other hand, the use of AE allowed us to obtain extracts with higher concentrations of squalene, α-tocopherol, β-sitosterol, and friedelin. From cell viability tests, the extracts are not considered toxic at the concentration tested (100 μg mg^{-1}).

Keywords: green solvent; ultrasound; extraction of active compounds; cytotoxicity; jambolan

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1. Introduction

Jambolan (*Syzygium cumini*), despite not being commercially produced, its parts have biological activity and can be used to obtain products with applications in the pharmaceutical and food industries. The leaves have a high phenolic content [1,2], with antidiabetic [3] and anti-inflammatory [1] effects. Flavonoids such as catechin and quercentin and phenolic acids such as gallic, caffeic, ferulic, ellagic, and p-coumaric were detected in the extract obtained from jambolan leaves [4].

The extraction of compounds from jambolan leaves normally occurs through the use of conventional techniques as performed by Veber et al. [5] and Misrahanum et al. [6], who obtained the phenolic compounds and antioxidants by maceration. Kaneria and Chanda [7] applied Soxhlet extraction; however, the focus of their study was the medicinal activity of the extract and not the intensification of extraction. Maceration is considered simple and suitable for protecting active compounds; however, it results in low extraction efficiency [8],

and Soxhlet extraction requires large amounts of time and solvent, in addition to contact with a high temperature and the possible degradation of the target compounds.

As an alternative to conventional techniques, ultrasound-assisted extraction (UAE) has been used, presenting high extraction efficiency and selectivity in short periods of time and mild temperatures, and requiring smaller volumes of solvent, as we can see in the recent results of different authors, in addition to maintaining the preserved biological activity of the extracts [9]. Mahindrakar and Rathod [10] reported the energy requirements and process costs for this technology, which were lower than those required by processes conducted in Soxhlet, such as sequential batch and stirred batch. This technology involves the use of ultrasonic waves that induce the phenomenon of cavitation, which causes a sequence of expansion and compression waves on the surface of the solid, causing the increase, collapse, and implosion of air bubbles, releasing energy in the form of waves [11].

Additionally, collapsing cavitation bubbles can rupture or remove the stagnant layer of solvent that forms around plant material and which acts as a diffusion barrier during the extraction process [12]. Mahindrakar and Rathod [10] used this technique to obtain the compounds present in *Syzygium cumini* leaves using water as a solvent. The study was focused on the effect of extraction variables on the removal of phenolic compounds and showed that the extract obtained had antioxidant, antidiabetic, and anticancer potential.

Hydroethanol extraction is commonly applied to obtain active compounds from vegetable leaves, whereas these solvents are food grade and non-toxic. As the ethanol concentration is reduced, the polarity index of the mixture increases, thereby increasing the solvation power [13], as well as the boiling point, facilitating the removal of target compounds [14]. While ethanol increases the solubility of the extracted species, the presence of water breaks the hydrogen bond between the matrix and the analytes [15].

The objective of this study was to employ UAE in order to obtain phytochemical extract from jambolan leaves using hydroethanolic solvent. For this purpose, the response surface methodology was applied to verify the effect of process variables (temperature, extraction time, and solvent to leaf ratio) on extraction mass yield (EMY) and phenolic compound yield (PCY) and establish the ideal region that maximizes these responses. The influence of the ethanol content in the extracting solvent was also evaluated on the composition of the extracts, and the results obtained were subjected to principal component analysis. Finally, the extracts were characterized in relation to phenolic compounds, flavonoids, active compounds, and their antioxidant, antidiabetic, and antibacterial activities. The cell viability of the extracts was also evaluated.

2. Materials and Methods

2.1. Raw Material

Syzigium cumini leaves obtained in Umuarama (Paraná, Brazil) (3°47′55″ S and 53°18′ 48″ W) were identified and deposited in an exhibition specimen in the herbarium of the State University of Maringá (registration HUEM 40310). The leaves were sanitized, the central stalk was removed and subsequently dried at 60 °C (Marconi, MA035, Piracicaba, Brazil) for 4 h, reaching a humidity of 4.38 ± 1.33 wt%. After drying, the material was crushed in a multiprocessor (Walita, RI7625, Itapevi, Brazil) and classified on Tyler-type sieves (Bertel, series 1.0, Caieiras, Brazil) to obtain particles with an average diameter of 0.557 mm.

2.2. Solvents, Reagents, and Analytical Standards

Ethanol (Honeywell™, Charlotte, NC, USA, purity ≥ 99.9% purity) and ultrapure water obtained from the Milli-Q purification system (Merck, Burlington, MA, USA) were used as solvents. For the analyses, the following were used: Folin–Ciocalteu (Dynamic, Indaiatuba, Brazil), gallic acid (Sigma-Aldrich St. Louis, MO, USA, 99.9%, purity), sodium carbonate (Anidrol, Diadema, Brazil), methanol (Neon, Suzano, Brazil), aluminum chloride (Dynamic), potassium acetate (Synth, Diadema, Brazil), distilled water, 2.2-diphenyl-1-picrylhydrazyl (DPPH•) (Sigma-Aldrich), 2.4.6-Tris(2-Pyridyl)-S-Triazine (Sigma-Aldrich),

trolox ((±)-6-hydroxy-2.5.7.8-tetramethylchromanwe-2-carboxylic acid (Sigma-Aldrich), hydrochloric acid (Anidrol), sodium acetate (Synth), glacial acetic acid (Anidrol), ferric chloride (Scientific Exodus, Sumaré, Brazil), methanol and formic acid suitable for HPLC (Merck), and chromatographic standards of squalene, α-tocopherol, β-sitosterol and friedelin (Sigma-Aldrich). To evaluate enzyme inhibition, α-glucosidase (Sigma-Aldrich), p-Nitrophenyl α-D-glucopyranoside (Sigma-Aldrich), α-amylase (Sigma-Aldrich), and soluble starch (Synth) were used.

In the antibacterial assays, the following were used: Brain Heart Infusion (BHI) broth (Kasvi, São Jose dos Pinhais, Brazil), Tween 80 (Sigma-Aldrich), 2.3.5-triphenyl-tetrazolium chloride (Sigma-Aldrich), and strains of *Staphylococcus aureus* (ATCC 12026), *Pseudomonas aeruginosa* (ATCC 9027), and *Escherichia coli* (ATCC 25922). For cell viability analysis, HaCaT cells, dulbecco's modified eagle medium (DMEM, Life Technologies, Waltham, MA, USA), fetal bovine serum (Gibco, São Paulo, Brazil), penicillin (Nova Biotecnologia, São Paulo, Brazil), streptomycin (Gibco), and MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, Sigma-Aldrich) were used.

2.3. Ultrasound-Assisted Extraction

Ultrasound-assisted extraction was conducted applying a Box–Behnken experimental design with three variables (temperature—T, extraction time—ET and solvent to leaf ratio—SLR) and levels (T—30, 45, and 60 °C; ET—10, 20, and 30 min; SLR—5, 10, and 15 mL g^{-1}).

In each experiment, ~3 g of sample was transferred to Erlenmeyer (250 mL), with the addition of hydroethanolic solvent (75% $v{\cdot}v^{-1}$ ethanol), which was positioned in the center of the ultrasonic bath (Ultronique, Q 5.9/40 A/165 W, Eco-Sonics, Indaiatuba, Brazil). The extraction and obtaining of the dry extract were conducted as reported by Raspe et al. [16] and the extraction mass yield (EMY) was obtained considering the mass of dry extract obtained and the initial mass of the leaf used.

To quantify the content of phenolic compounds, the Folin–Ciocalteau method [17] was adopted from the solubilization of the extract in the extraction solvent. The absorbance was determined in a spectrophotometer (Shimadzu, UV-1900, Japan, Tokyo) at 760 nm and to quantify the content of phenolic compounds, a standard curve prepared with a standard gallic acid solution was used. The phenolic compounds yield (PCY) was determined from the content in the extract and the EMY; the result was expressed in mg equivalent to GAE g^{-1} of leaf.

Analysis of variance (ANOVA) used Statistica® 8.0 software (StatSoft, Inc., Tulsa, OK, USA) to evaluate the effects of independent variables (with 95% confidence interval) on responses and experimental data were adjusted to a second-order polynomial model.

To determine the conditions that maximize the responses, in the considered experimental range, the Derringer desirability function was applied, and the predictive capacity of the polynomial model was evaluated by verification experiments conducted under this condition. In the experimental condition of maximum extraction, solvents with the following different compositions were tested: absolute ethanol (AE), 75:25 ($v{\cdot}v^{-1}$) ethanol:water (75E), and 50:50 ($v{\cdot}v^{-1}$) ethanol:water (50E).

2.4. Extract Characterization

The phenolic compounds content was determined as described in Section 2.3. The aluminum chloride colorimetric method [18] was used to determine the flavonoid content from a standard curve prepared with quercetin.

The identification of active compounds was determined in a triple quadrupole mass spectrometer (model XEVO TQD from Waters, Milford, MA, USA) equipped with an electrospray ionization (ESI) source operating in negative ionization modes [2]. Data were collected and processed with MassLynx software (version 4.1) and the Pubchem database was used to identify the compounds.

For CG-FID analysis, the extracts were diluted in ethanol and kept at 60 °C for 15 min. The solution was filtered and then injected (1 µL) into a NA-5 capillary column (Analytical,

5% Phenyl–Methylsiloxane, 30 m × 0.25 mm id, 0.25 µm) at a split rate of 1:30. The initial temperature of the column was 185 °C, which was subsequently increased by 6 °C min^{-1} until reaching 300 °C, remaining at this temperature for 8 min. The injector and detector temperatures were maintained at 280 °C and 300 °C, respectively. The compounds were identified by comparing the retention time of the chromatographic standards and quantification was carried out using calibration curves obtained by injecting solutions of these at different concentrations.

To determine the antioxidant potential, the DPPH• free radicals and the iron reduction method (FRAP) as described by Gu et al. [19] and Rufino et al. [20], respectively, were used.

The results were evaluated by ANOVA using Statistica® 8.0 software, followed by a comparison of the means using the Tukey test (with 95% confidence interval).

2.5. Inhibition of Enzymes and Antibacterial Activity

Inhibition of α-glucosidase and α-amylase were performed according to Kim et al. [21] (Equation (1)). In both analyses, the samples were diluted in 0.1 mol L^{-1} potassium phosphate buffer (pH 6.8) to obtain a concentration of 0.25 mg mL^{-1}. A solution without the extract was used as a control, and a solution without the substrate was used as a blank.

$$\text{Inhibition } (\%) = (1 - (\text{Abs sample} - \text{Abs blank}) / \text{Abs control}) \times 100 \quad (1)$$

The antibacterial activity was analyzed in independent duplicates by a serial microdilution method in 96-well reservoir microplates [22]. The test was carried out using the methodology applied by Pinc et al. [23].

2.6. Cell Viability Assay

To evaluate cell viability, HaCat cells were used. Samples of the extracts (100 µg mg^{-1}) were diluted in the culture medium and incubated in a shaker for 48 h (room temperature). Then, 200 µL of this solution was added to each well. Subsequently, the culture medium was removed, the MTT solution (5 mg mL^{-1}) was added, and the presence of formazan crystals was evaluated using a microplate reader (Agilent, Santa Clara, CA, USA) at 550 nm. For the execution of the test, a negative control (without extract), a vehicle control (70% alcoholic), and a positive control (with hydrogen peroxide) were performed, and the cell viability was calculated according to Malich et al. [24].

3. Results and Discussion

3.1. Experimental Design: Establishment of Predictive Equations

Table 1 presents the results obtained from EMY and PCY in the experimental runs proposed by the experimental design.

Table 1. Experimental conditions and results of extraction mass yield (EMY) and phenolic compounds yield (PCY).

Run	Variable [1]			EMY (wt%)	PCY [2] (mg GAE g^{-1} Leaf)
	T	ET	SLR		
1	−1 (30)	−1 (10)	0 (10)	6.03	4.19 ± 0.07
2	1 (60)	−1 (10)	0 (10)	8.04	7.27 ± 0.07
3	−1 (30)	1 (30)	0 (10)	7.39	5.64 ± 0.04
4	1 (60)	1 (30)	0 (10)	9.44	10.41 ± 0.12
5	−1 (30)	0 (20)	−1 (5)	5.88	3.48 ± 0.15
6	1 (60)	0 (20)	−1 (5)	7.52	7.22 ± 0.59
7	−1 (30)	0 (20)	1 (15)	8.25	6.25 ± 0.67
8	1 (60)	0 (20)	1 (15)	9.99	13.37 ± 0.16
9	0 (45)	−1 (10)	−1 (5)	4.68	4.12 ± 0.02
10	0 (45)	1 (30)	−1 (5)	6.55	6.55 ± 0.05

Table 1. *Cont.*

Run	Variable [1]			EMY (wt%)	PCY [2] (mg GAE g^{-1} Leaf)
	T	ET	SLR		
11	0 (45)	−1 (10)	1 (15)	8.58	5.17 ± 0.05
12	0 (45)	1 (30)	1 (15)	8.92	9.31 ± 0.08
13–16 [3]	0 (45)	0 (20)	0 (10)	8.53 ± 0.43	8.72 ± 0.47

[1] T: temperature (°C); ET: extraction time (min); SLR: solvent to leaf ratio (mL g^{-1}). [2] GAE: gallic acid equivalent. [3] Mean of four repetitions.

Based on the results in Table 1, the analysis of variance (Table 2) was generated to evaluate the effects of each variable (linear and quadratic) as well as their interactions in the response variables.

Table 2. Analysis of variance of regression models for extraction mass yield (EMY) and phenolic compound yield (PCY).

	EMY				PCY			
Factor	**Sum of Squares**	**Mean Square**	**F**	***p*** [a]	**Sum of Squares**	**Mean Square**	**F**	***p*** [a]
(T) (L)	6.94	6.94	37.96	<0.01	45.19	45.19	207.70	<0.001
(T) (Q)	0.02	0.02	0.12	0.76	0.30	0.30	1.39	0.323
(ET) (L)	3.09	3.09	16.91	0.03	15.58	15.58	71.59	0.003
(ET) (Q)	2.58	2.58	14.10	0.03	10.77	10.77	49.52	0.006
(SLR) (L)	15.44	15.44	84.47	<0.01	21.24	21.24	97.62	0.002
(SLR) (Q)	1.52	1.52	8.34	0.06	2.98	2.98	13.71	0.034
TxET	<0.01	<0.01	<0.01	0.96	0.71	0.71	3.28	0.168
TxSLR	0.003	<0.01	0.01	0.91	3.39	3.39	15.57	0.030
ETxSLR	0.57	0.58	3.16	0.17	0.73	0.73	3.35	0.164
Lack of fit	0.35	0.12	0.64	0.64	5.53	1.84	8.47	0.056
Pure Error	0.55	0.18			0.65	0.22		
Total	31.06				107.07			

[a] Statistical significance ($p < 0.05$); L: linear effect; and Q: quadratic effect.

From the data presented in Table 2, it can be observed that the linear terms of all variables had an effect for EMY and PCY. Among the quadratic terms, the time variable had an effect for both responses and the solvent to leaf ratio variable had an effect only for PCY. The interaction between temperature and solvent to leaf ratio had an effect for PCY. Based on the significant terms, the polynomial equations adjusted to the experimental data were obtained as follows (Equations (2) and (3)):

$$\text{EMY} = 7.89 + 0.89\ \text{T} + 0.59\ \text{ET} + 1.33\ \text{SLR} - 0.77\ \text{ET}^2 \quad (2)$$

$$\text{PCY} = 8.66 + 2.38\ \text{T} + 1.40\ \text{ET} + 1.63\ \text{SLR} - 1.64\ \text{ET}^2 - 0.86\ \text{SLR}^2 + 0.92\ \text{T} \times \text{SLR} \quad (3)$$

The generated polynomial models are considered valid in relation to the experimental data, considering the non-significant lack of fit *p*-value and the values obtained from Fcalc (25.91) > Ftab (3.36) for EMY and Fcalc (13.61) > Ftab (3.37) for PCY. Additionally, the generated diagnostic graphs (Supplementary Materials) confirm the adequacy of the model and the validity of its predictions. Figures S1a and S2a show that the residual values were less than 1, with no discrepancies between the values or unexpected errors. In Figures S1b and S2b the agreement between the experimental and predicted data are verified ($R^2 > 0.90$).

3.2. Analysis of Effects

Figure 1 presents the three-dimensional graphs used to represent the effects of the variables and their interactions according to Equations (2) and (3) (each graph is a function of two variables, keeping the third at the central point).

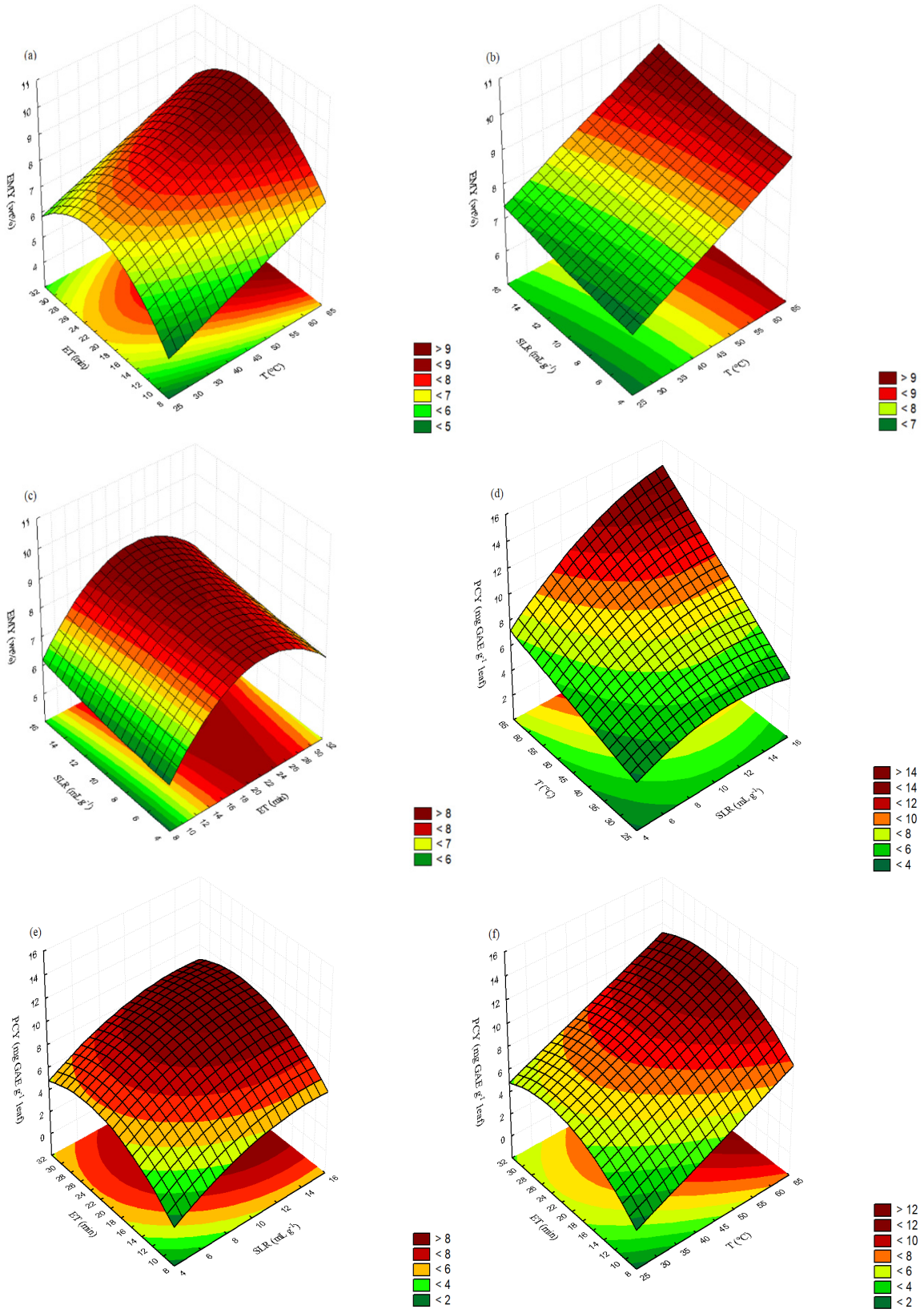

Figure 1. Response surface for extraction mass yield (EMY) and phenolic compound yield (PCY). Correlative effects for EMY: (**a**) time and temperature, (**b**) solvent to leaf ratio and temperature, and (**c**) solvent to leaf ratio and time. Correlative effects for PCY: (**d**) temperature and solvent to leaf ratio, (**e**) time and solvent to leaf ratio, and (**f**) time and temperature.

Figure 1a shows an increase in EMY with a progressive increase in T, while for ET, a slight decrease in EMY can be observed to a certain extent. The interaction between T and SLR in Figure 1b resulted in higher EMY. Figure 1c showed an increase in the dependent variable in favor of an increase in ET and SLR. Figure 1d showed a greater influence of SLR and T for PCY, obtaining up to >14 mg GAE g^{-1} leaf. The upward behavior of SLR and ET resulted in obtaining higher PCY contents (Figure 1e). The application of high T values favored PCY in a short ET period (Figure 1f).

3.2.1. Effect of Temperature

The observed effect of temperature is due to the reduction in viscosity and surface tension between the solute and solvent with the application of higher values of this variable, which consequently increases the penetration of the solvent into the matrix [25] and the diffusion of molecules in the extraction medium [26]. In addition, it causes the softening of plant tissues and accelerates the dissolution of active compounds from the leaves [27]. Jo and Kim [28] observed an increase in the effective diffusion coefficient from 1.281×10^{-13} to 5.977×10^{-13} m^2 s^{-1} as the extraction temperature was raised from 25 to 45 °C, indicating that the external resistance to mass transfer is negligible due to the efficient mixing of solute and solvent.

The increase in the solubility of phenolic compounds in the solvent with the increase in extraction temperature and reduction in solvent viscosity contributes to the greater recovery of these compounds from the plant matrix [29,30]. Additionally, the increase in the diffusion coefficient and mass transfer coefficient with increasing temperature in the experimental range close to the values applied in this study is reported. Sharma and Dash [31] reported an increase in the values of the diffusion coefficient from 5.704 m^2 s^{-1} to 10.515 m^2 s^{-1} with an increase in temperature from 40 to 70 °C, respectively. Raja and Dash [32] found that increasing the temperature from 30 to 60 °C increased the effective diffusion coefficient from 2.988 ± 0.015 m^2 s^{-1} to 4.841 ± 0.020 m^2 s^{-1} and mass transfer coefficient from 2.004 ± 0.015 m s^{-1} to 2.807 ± 0.012 m s^{-1}. It is also evident from the thermodynamics analysis that the phenolic extraction process is spontaneous and feasible due to the negative values of the variation in Gibbs free energy, which become more negative with the increasing of the temperature [33,34].

3.2.2. Effect of Time

The favoring of EMY linked to the increase in extraction time is due to the high mass transfer rate caused by the driving force of effortless acoustic cavitation [35]. Frohlich et al. [36] observed that clove leaf extract increased with longer application, which may have occurred in two periods, consisting of a superficial wash in the first period, in which the soluble compounds of the vegetative surface are extracted. The second period involves the transfer of mass from the interior of the matrix to the solvent by the phenomenon of diffusion [37].

Increasing the time from 10 to 30 min favored PCY. However, when observing the results obtained at 30 °C and with SLR of 10 mL g^{-1}, when increasing the time from 10 to 30 min, there was an increase of ~34% in PCY, which can be explained by the total removal of the extract in the first washing step. Sahin and Shamli [38] identified that 20 to 40 min of ultrasound removal of the olive leaf extract increased the number of phenolic compounds by ~17%, which is explained by the two washing stages, in which the first involves the dissolution of soluble compounds on the surfaces of the samples, called "washing", followed by the diffusion of the solute into the solvent.

3.2.3. Effect of Solvent to Leaf Ratio (SLR)

The SLR variable had a greater influence on EMY, as can be seen in the comparison of the results of runs 5 and 7, 6 and 8, 9 and 11, and 10 and 12 (Table 1). This effect is due to the increase in the concentration gradient, which in turn increases the mass transfer rate and consequently the extraction efficiency [39,40].

The extraction of phenolic compounds was also favored by the addition of solvent to the extraction medium. Wang et al. [41] reported that mass transfer from solid to solvent depends on their proportion, as an increase in solvent resulted in a more effective rate. In addition, a large amount of cavitation bubbles was formed when a greater proportion of solvent was applied to the extraction in relation to the solid. Shewale and Rathod [33] obtained diffusion coefficient values of 1.869 and 2.026 $\times 10^{-12}$ m^2 s^{-1} by increasing the solid to liquid ratio from 1:10 to 1:20, respectively.

3.3. Verification Experiments

From the predicted equations (Equations (2) and (3)), the conditions that maximize EMY (9.94 wt%) and PCY (13.01 mg GAE g^{-1} leaf) were determined, which presented a desirability factor of 1.00 and 0.955, respectively; T = 60 °C, ET = 30 min, and SLR = 1:15 g mL^{-1}. Verification experiments conducted under these conditions resulted in 9.99 ± 0.42 wt% and 13.67 ± 0.22 mg GAE g^{-1} leaf for EMY and PCY, respectively, which do not differ from the predicted values ($p > 0.05$).

3.4. Effect of Extractor Solvent Composition

3.4.1. Extraction Mass Yield

The use of AE, 75E, and 50E as extraction solvents led to obtaining EMY of 4.33 ± 0.42 wt%, 9.99 ± 0.42 wt%, and 8.92 ± 0.25 wt%, respectively. These results are due to the increase in solvent polarity and the ability to dissolve more compounds with the increase in water content [42,43]. The dielectric constant also changes as the water concentration increases, which increases the extraction of polar compounds [44]. Mokaizh et al. [45] reported that the addition of water to ethanol has two effects, the first of which increased the permeability of the plant matrix, facilitating the process of extract removal, and the second disturbed the relationship between the solutes and the plant matrix. Additionally, the constituents of jambolan leaves belong to the class of polar compounds [46].

3.4.2. Composition of Dry Extracts

Table 3 presents the results obtained from the composition of the extracts resulting from the use of different solvents. As can be seen in this table, under the experimental conditions evaluated, the extracts presented higher levels of phenolic compounds and flavonoids for 50E. As the amount of water added to the ethanol increases, the extraction efficiency can improve, as it increases the removal of the phenolic content from the samples. This occurs because the polarity of the mixture increases and consequently the solubility of the extracts in the solvent [9], facilitating diffusivity due to the reduction in the dielectric constant of the solvent [47]. The phenolic composition of the three extracts obtained from jambolan leaves revealed the presence of some phenolic acids and quercetin. The composition of the solvent used directly influenced the intensity of the compounds obtained, which were generally greater when hydroethanolic solvents (18% and 56%) were applied, compared to using AE.

The antioxidant potential showed similar behavior to phenolic compounds and flavonoids, with higher values obtained using 50E as solvent, suggesting that these compounds are responsible for the determined antioxidant potential. Studies confirm the radical scavenging activity of trans-caffeic acid in relation to DPPH•, that is, it is influenced by the number of hydroxyls in the aromatic ring; the greater the number of hydroxyl groups, the greater the radical scavenging activity [48]. The antioxidant potentials of sinapic, vanillic, and quinic acids, also identified in higher intensities in the samples, were reported by Subramanian [49], Tai et al. [50], and Karaman et al. [51], respectively.

Squalene, α-tocopherol, β-sitosterol, and friedelin were identified in the extracts obtained. The squalene content was quantified ~3000% higher in AE compared to 50E, which can be justified by the solvent characteristic; the AE extract presented a nonpolar characteristic compared to 50E, as the squalene molecule has hydrophobic properties [52]. Fernandes et al. [53] obtained high concentrations of squalene (23.28%) in extracts of

Echinodorus macrophyllus using apolar solvent. This is consistent with the other compounds, α-tocopherol, β-sitosterol, and friedelin. This study stated that AE is a good solvent for the removal of apolar compounds, in agreement with the findings reported by Milovanovic et al. [54], who detected a greater number of tocopherols in ethanolic extracts obtained from dandelion seeds. According to a study carried out by Ravi et al. [55], the compound β-sitosterol presents a hydrophobic and nonpolar behavior. The nucleus of this compound can repel or avoid water molecules, which is confirmed in the results of this work. Vieira et al. [56] identified the compound friedelin in Quercus cerris extracts and observed that the highest concentration of this compound was in tests involving weakly polar or nonpolar solvents.

Table 3. Characterization of dry extracts obtained with the application of solvents with different compositions.

Property		AE	Hydroethanolic Solvent	
			75E	50E
Phenolic compounds (mg GAE g^{-1} extract)		55.81 ± 1.6 [a]	136.86 ± 1.61 [b]	196.27 ± 1.48 [c]
Flavonoids (mg QE g^{-1} extract)		112.02 ± 0.90 [a]	190.35 ± 2.75 [b]	204.80 ± 1.99 [c]
Compounds identified by UPLCLC-MS/MS (Intensity per sample concentration)/10^9	Gallic acid	7.64	5.54	5.70
	Sinapic acid	26.58	26.77	45.05
	trans-caffeic acid	4.40	6.67	12.95
	p-Coumaric acid	2.33	3.00	3.24
	Vanillic acid	7.64	13.23	15.30
	Syringic acid	1.57	1.31	1.98
	Quercetin	3.61	2.90	2.66
	Quinic acid	9.35	16.00	13.75
	Ellagic acid	2.83	2.61	2.43
Compounds quantified by GC-FID (mg 100 g^{-1} extract)	Squalene	161.38 ± 0.36 [a]	45.82 ± 0.12 [b]	5.12 ± 0.10 [c]
	α-tocopherol	124.28 ± 4.72 [a]	40.36 ± 0.50 [b]	8.32 ± 0.64 [c]
	β-sitosterol	480.83 ± 14.61 [a]	196.60 ± 0.53 [b]	42.47 ± 5.20 [c]
	Friedelin	311.68 ± 9.39 [a]	72.00 ± 0.74 [b]	10.08 ± 0.18 [c]
Antioxidant potential (μmol TEAC g^{-1} extract)	DPPH•	531.97 ± 4.83 [a]	708.67 ± 9.43 [b]	832.82 ± 14.50 [c]
	FRAP	479.32 ± 9.05 [a]	619.00 ± 9.43 [b]	724.00 ± 7.07 [c]

GAE: gallic acid equivalent; QE: Quercetin equivalent. TEAC: Trolox equivalent antioxidant capacity; AE: Absolute ethanol. 75E: 75% $v{\cdot}v^{-1}$ ethanol; 50E: 50% $v{\cdot}v^{-1}$ ethanol. Means followed by different letters on the same line indicate a significant difference ($p < 0.05$).

3.4.3. Inhibition of Enzymes

Table 4 shows the inhibition of the enzymes α-glucosidase and α-amylase that expresses the antidiabetic activity of jambolan leaf extracts. As observed, as the water concentration increased, there was also an increase in the inhibition of the α-amylase enzyme. For α-glucosidase, the concentration of ethanol in the solvent, in the tested extract concentration, did not interfere with the inhibition of this enzyme.

Table 4. Antidiabetic activity of jambolan leaf extracts.

Solvent	(% Inhibition)	
	α-Amylase	α-Glucosidase
AE	26.90 ± 3.42 [a]	99.76 ± 0.09 [a]
75E	39.04 ± 3.01 [b]	99.65 ± 0.12 [a]
50E	37.52 ± 2.33 [b]	99.47 ± 0.25 [a]

AE: Absolute ethanol; 75E: 75% $v{\cdot}v^{-1}$ ethanol; 50E:50% $v{\cdot}v^{-1}$ ethanol. Means followed by different letters on the same column indicate a significant difference ($p < 0.05$).

The observed effects may be due to the higher concentrations of phenolic and flavonoid compounds in the extracts, as well as the constituents identified by GC/FID. Kwon et al. [57] indicate that the content of flavonoids and polyphenols in plant extracts has strong inhibitory effects on α-amylase and α-glucosidase. Han et al. [58] show that quinic acid presented a 40% inhibition of α-glucosidase 1.05 mg mL^{-1} and Aleixandre et al. [59] obtained 60% and 80% inhibitions of this enzyme with vanillic and syringic acids at concentrations of 1.38 mg mL^{-1} and 1.78 mg mL^{-1}, respectively. Additionally, Smruthi et al. [3] found that the compounds friedelin, β-sitosterol, and quercetin showed inhibition of α-amylase. Kumar et al. [60] obtained from molecular docking that friedelin is a promising candidate for developing new antidiabetic inhibitors targeting α-glucosidase and α-amylase.

3.4.4. Antibacterial Activity

Table 5 presents the results of the antibacterial activity of the extracts obtained, in which it is observed that bacterial growth was inhibited at the same concentrations for *E. coli.* For *S. aureus*, the MIC values did not differ between the samples obtained with the solvents AE and 75E; however, this strain was the most susceptible to the extract obtained at 50E. *P. aeruginosa* presented the same MIC for extracts 75E and 50E, which was lower than that obtained using AE.

Table 5. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) for jambolan leaf extracts.

	Microorganisms	**AE**	**Hydroethanolic Solvent**	
			75E	**50E**
MIC/MBC (μg mL^{-1})	*Escherichia coli*	31.25 ± 0.00/>500	31.25 ± 0.00/>500	31.25 ± 0.00/>500
	Staphylococcus aureus	31.25 ± 0.00/125	31.25 ± 0.00/>500	7.81 ± 0.00/125
	Pseudomonas aeruginosa	62.50 ± 0.00/125	31.25 ± 0.00/>500	31.25 ± 0.00/125

AE: Absolute ethanol. 75E: 75% $v{\cdot}v^{-1}$ ethanol. 50E:50% $v{\cdot}v^{-1}$ ethanol.

Jassim et al. [61] determined MICs for jambolan leaf extracts of 208, 208, and 104 μg mL^{-1} of *E. coli*, *P. aeruginosa* and *S. aureus*, respectively. Oliveira et al. [62] reported MIC values of 200, 200, and 90 μmg mL^{-1} when the hydroalcoholic extract of *Syzygium cumini* leaves was applied to strains of E. coli, *S. aureus*, and *P. aeruginosa*, respectively, and indicated that the extract has potential as a topical antibacterial therapy to promote the healing of skin wounds. In this study, hydroalcoholic extracts were also used; however, the results obtained were superior to those reported in the literature, which can be explained by the concentration of the ethanol and water mixture used.

The antibacterial activity present in this plant may be due to the presence of tannins and other phenolic constituents [62]. Engels et al. [63] indicate the inhibitory effect of sinapic acid against Gram-positive and Gram-negative bacteria. Bai et al. [64] demonstrated that quinic acid is capable of interacting with the cell membrane of *S. aureus*, leading to the dysfunction of oxidative phosphorylation, which favors the use of this acid as a potential antibacterial agent. This can be explained by the degree of polarity of the extracts with 50% ethanol and water and the polarity of the membranes of Gram-negative bacilli [65].

3.4.5. Cell Viability

The cell viability results (Figure 2) indicated that the extracts obtained are non-toxic, despite the samples AE and 75E differing significantly from each other. However, all samples showed cell viability > 90%. The extract produced with 75E and 50E showed an increase in cell viability, suggesting possible cell proliferation compared to the extract obtained with AE, which may be related to the presence of a higher number of bioactive compounds in the extract (Table 2). According to ISO 10993-5 [66], cell viability values lower than 70% indicate cytotoxic potential. Therefore, the extracts are considered safe at the tested concentration. Santos et al. [67] valuated the cytotoxicity of freeze-dried jambolan

leaves and reported that, at the tested concentrations (0.1 to 10 μg/mL), the extract did not affect cell viability according to the MTT assay results, indicating that the extract is considered non-toxic.

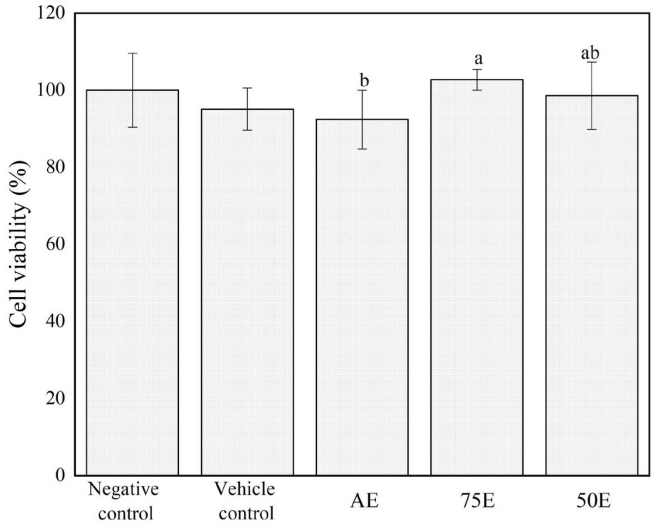

Figure 2. Results of cell viability. AE: absolute ethanol; 75E: 75% $v{\cdot}v^{-1}$ ethanol; and 50E: 50% $v{\cdot}v^{-1}$ ethanol. Means followed by different letters indicate a significant difference ($p < 0.05$) for the different extracts tested (AE, 75E, and 50E).

4. Conclusions

The preparation of extracts with a high phenolic content was effectively carried out using the selected extraction technique and considering the evaluated process parameters. The leaf to solvent ratio had a greater influence on obtaining extract mass and the temperature on the extraction of phenolics. The time variable had little influence on the evaluated responses, possibly due to greater extraction in the initial washing stage. Therefore, it was possible to obtain 9.94 wt% of extract from the leaves with 136.86 mg GAE per g under maximized conditions. The addition of water to the solvents had a notable effect on the antioxidant capacity of the extracts, with an increase of ~50%. Based on the composition of the extracts obtained, it is evident that the use of a solvent with a higher water content allowed for greater removal of phenolic acids and flavonoid compounds. However, on the other hand, an extract with a higher concentration of liposoluble compounds can be obtained with the use of absolute ethanol. Finally, extracts obtained did not show a cytotoxic effect, which was evaluated by cell viability tests. These findings demonstrate the potential of jambolan leaves as a viable option for obtaining valuable compounds for functional food, nutraceutical, cosmetic, and pharmaceutical applications due to their composition and antioxidant potential. Therefore, future research should focus on its applicability, as well as on the purification of the extract aiming to enhance its phytochemical characteristics.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/pr12102270/s1; Figure S1. Model diagnostic charts for extraction mass yield (EMY): (a) Raw Residual normal probability plot and (b) Predicted vs. observed values; Figure S2. Model diagnostic charts for phenolic compounds yield (PCY): (a) Raw Residual normal probability plot and (b) Predicted vs. observed values.

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