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Pressurized liquid extraction as a green technique for bioactive compounds recovery from *Momordica charantia* leaves

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ABSTRACT

Momordica charantia L., an unconventional food plant, has long been used in traditional medicine to treat conditions such as diabetes and digestive disorders. This study employed pressurized liquid extraction (PLE) to recover bioactive compounds from *M. charantia*, examining the effects of different extraction parameters, including solvent type (ethanol, 50 % hydroalcoholic mixture, water), temperature (40, 50, 60 °C), and solvent flow rate (1, 2, 3 g/min). The extracts were analyzed for global yield, total phenolic content (TPC), total flavonoid content (TFC), chlorophyll content, and antioxidant capacity (DPPH and ABTS assays). Phenolic compounds were identified through high-performance liquid chromatography coupled with electrospray ionization mass spectrometry (HPLC-DAD-ESI-MS^n). PLE using 50 % hydroalcoholic solvent at 60 °C yielded the highest TPC (14.4 ± 0.3 mg GAE/g sample) and TFC (6.6 ± 0.1 mg QE/g sample). Ethanolic PLE extracts exhibited higher antioxidant activity in the DPPH assay, while aqueous extracts showed greater activity in the ABTS assay, likely reflecting differences in chemical composition. A total of 24 phenolic compounds, including phenolic acids, flavanones, and flavonols, were identified using HPLC-DAD-ESI-MS^n, with profiles varying according to the solvent type used for PLE. In the modeling of PLE kinetics curves, the Spline model identified three distinct stages in the extraction process. Optimal conditions were determined to be a temperature of 60 °C and a solvent flow rate of 2 g/min, which maximized bioactive compound extraction. These results highlight the potential of PLE as an efficient method for valorizing *M. charantia* through the extraction of bioactive compounds.

1. Introduction

Unconventional Food Plants (UFP) are plants that are easily found and cultivated, requiring minimal agricultural inputs (Joseph et al., 2023). This classification includes fruit, medicinal, and cereal species that are rich in nutritional value and bioactive compounds. These plants are used for the development of new products and are associated with antioxidant capacity (de Souza et al., 2023; Pereira et al., 2023; Talucder et al., 2024). Thus, cultivating UFP emerges as an alternative to combating food insecurity and preserving biodiversity, as they offer low costs, the potential for dietary diversification, and income generation for small-scale farmers. This aligns with the United Nations' 2030 Agenda for Sustainable Development Goals (SDGs), particularly SDG 9 (Industry, Innovation, and Infrastructure) and SDG 12 (Responsible Consumption and Production) (de Souza et al., 2023; Milião et al., 2022; Vardanega et al., 2022).

Momordica charantia L., also known as bitter melon, due to its particular bitter taste, is a UFP from the Cucurbitaceae family. It is innate to subtropical and tropical areas in Asia and some other parts of the world, including Brazil. Morphologically, it is a herbaceous plant that grows up to 5 m in length, with simple, alternate leaves measuring 4–12 cm, divided into 3–7 deeply separated lobes (Saeed et al., 2018).

M. charantia has long been used in traditional medicine for the treatment of various illnesses, including diabetes, digestive disorders, chronic fever, skin diseases, joint pain, and others, due to the presence of primary and secondary metabolites (Grover & Yadav, 2004). The plant is rich in proteins and peptides, including glycopeptides like α- and β-momorcharins. These glycoproteins exhibit anti-tumor and immunomodulatory activities (Jia et al., 2017). Additionally, it also contains various polyphenols, especially in its leaves, including catechins, gallic

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acid, and chlorogenic acid, which are known for their strong antioxidant and anti-inflammatory properties. These compounds act as effective free radical scavengers and contribute to the plant's bioactivities, such as mitigating oxidative stress and inflammation in chronic diseases (Bora et al., 2023; Dah-Nouvlessounon et al., 2023).

Emerging extraction methods like ultrasound-assisted extraction, microwave-assisted extraction, pressurized liquid extraction (PLE) and supercritical fluid extraction can offer significant advantages, such as reduced extraction time, lower solvent consumption, and enhanced recovery of bioactive compounds, while being environmentally friendly (Khan et al., 2024). PLE method, characterized by the use of high pressures that allow operation using liquid solvents at elevated temperatures, facilitates increased diffusion, solubility of compounds, mass transfer, and thus, process improvement (Višnjevec et al., 2024). Additionally, PLE demonstrates particular efficiency in recovering phenolic compounds, especially when employing ethanol-water mixtures (de Aguiar et al., 2019).

The use of GRAS (Generally Recognised as Safe) solvents, as ethanol and water, presents an excellent alternative for recovering bioactive compounds from UFP. These solvents are non-toxic to the environment, making them ideal for applications in various industries, including pharmaceuticals, cosmetics, and food industries (López-Salas et al., 2024). Particularly within the food industry, bioactive compounds extracted from UFP can serve as flavor enhancers, prevent lipid oxidation in meat products, extend shelf life, among others (Iqbal et al., 2022).

The objective of this study was to investigate the influence of PLE parameters, including solvent type, temperature, and flow rate, on the extraction yield, bioactive compound content (total phenolics, flavonoids, and chlorophyll), phenolic profile (analyzed by HPLC-DAD-ESI-MS^n), and antioxidant capacity (ABTS and DPPH assays) of *M. charantia* leaves. Additionally, the study assessed the effect of temperature and flow rate on extraction kinetics using ethanol, a 50 % hydroalcoholic solution, and water at temperatures of 40, 50, and 60 °C, with flow rates of 1, 2, and 3 g/min.

2. Material and methods

2.1. Raw material characterization

M. charantia leaves were collected in the municipality of Aragarças - Goiás (15° 53 ʾ42.9 "S 52° 14′ 15.4″W) in December 2022. The leaves were dried (room temperature "30 °C" for 48 h), ground using an analytical mill (IKA, model A11 basic mill, Brazil), and stored at -18 °C for subsequent steps. Moisture + volatile content was determined AOAC 930.04 (A.O. A.C, 1997). The moisture content was determined by the Karl Fisher volumetric method (Titrino Plus, model 870, Metrohm, Switzerland) equipped with a Thermoprep KF oven (Metrohm model 832, Switzerland) (A.O.C.S, 2009). The mean particle diameter was estimated by the ASAE method (ASAE, 1997), employing a vibratory sieve system (Model 1868, Bertel, SP, Brazil), from the means of the materials retained on the sieve meshes 16, 24, 32, 48, 60 and 100. The apparent density of the particle bed was determined through the mass-to-volume ratio.

2.2. Chemicals

Ethanol (99.8 %, Dinâmica, Brazil) and distilled water were used as solvent. Gallic acid (Dinâmica, Brazil), Folin-Ciocalteu reagent (Êxodo, Brazil), quercetin (Cayman Chemical, USA), 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS, 99 %) (Sigma-Aldrich, Brazil), 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma-Aldrich, Brazil), (±)− 6-hidroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox®) (Sigma-Aldrich, Brazil) were used in analytical methods.

2.3. Extraction processes

The sample, which consisted of dried and ground *M. charantia* leaves were subjected to the PLE and Soxhlet extraction processes as described below.

2.4. Pressurized liquid extraction (PLE)

Pressurized liquid extraction experiments were conducted in an experimental unit (UFMT, Brazil) schematically depicted in Fig. 1, which was used in the studies by Paula et al. (2013), with some modifications.

The stainless steel AISI 316 L extractor with a volume of 50 mL was packed manually with approximately 5 g of sample forming a bed of particles, and the remaining volume of the extractor was filled with glass beads. The extraction parameters evaluated were extraction solvent, temperature and solvent flow rate. The temperatures were 40, 50 and 60 °C, the solvents used were ethanol ($\rho = 0.78$ g/mL), hydroalcoholic 50 % (v/v) ($\rho = 0.81$ g/mL) and distilled water ($\rho = 1.0$ g/mL) and flow rates of 1 g/min; 2 g/min and 3 g/min. In PLE, due to the incompressibility of liquids, changes in density with pressure are generally minimal (Mustafa & Turner, 2011), thus pressure was kept constant at 12 MPa, and total extraction time was 70 min.

The solvent-to-sample ratio (S/F) varied across the different extraction conditions. S/F values for the PLE using ethanol as solvent were 10.9, 21.8 e 32.7 for 1 g/mL, 2 g/mL and 3 g/mL, respectively. For hydroalcoholic solvent the S/F values were 11.3, 22.7 and 34 for 1 g/mL, 2 g/mL and 3 g/mL, respectively. For water as solvent the S/F values were 14, 28 and 42 for 1 g/mL, 2 g/mL and 3 g/mL, respectively. All experiments were performed in duplicates.

2.5. Soxhlet extraction

For Soxhlet extraction, 5 g of the sample were packed in filter paper and placed in the extractor with 200 mL of solvent (S/F = 40). Ethanol and water were used as solvents. The extraction was conducted following the method described by de Aguiar et al. (2019). The system was heated to the boiling point of the solvent and maintained under reflux for 6 h. All experiments were performed in triplicate.

2.6. Global extraction yield (X_0)

Global extraction yield was calculated to compare the efficiency among the different extraction methods and represents the relation between the mass of extract (m_{ext}) and sample (m_{sample}), as described in Eq. 1.

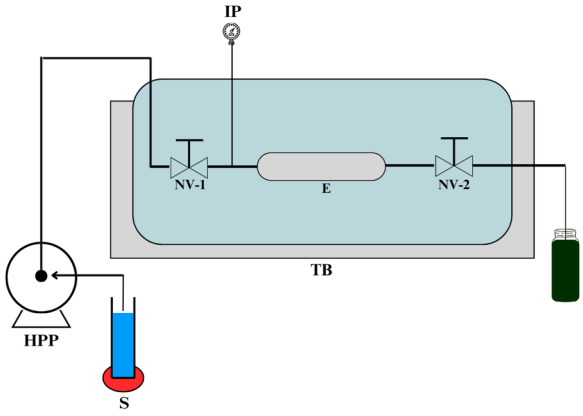


Fig. 1. Experimental unit of pressurized liquid extraction (HPP) high pressure pump, (S) solvent, (TB) thermostated bath, (NV-1 and NV-2) needle valves, (IP) Bourdon pressure indicator and (E) extractor.

$$X_0(\%) = \frac{m_{ext}}{m_{sample}} \times 100 \quad (1)$$

2.7. PLE kinetics and modeling

The kinetic experiments were carried out with 5 g of sample, at a fixed pressure of 12 MPa, for the solvent hydroalcoholic 50 % at temperatures of 40, 50 and 60 °C, and solvent flow rates of 1, 2 and 3 g/min. Each experimental condition was performed in duplicate. The PLE extraction curves were obtained by plotting the global yield versus extraction time.

The three-line Spline model, presented in Eq. (2) to (4), was fitted to the experimental data using the PROC NLIN procedure of the SAS University Edition software, as described by Rodrigues et al. (2002). Each fitted line represents an extraction stage related to a mass transfer mechanism: constant extraction rate (CER), falling extraction rate (FER) and diffusion-controlled periods (DC), as described by Pereira e Meireles (2010).

$$Y = b_0 + b_1 t \text{ for } t \leq t_{CER} \quad (2)$$

$$Y = b_0 - t_{CER} b_2 + (b_1 + b_2) t \text{ for } t_{CER} < t \leq t_{FER} \quad (3)$$

$$Y = b_0 - t_{CER} b_2 - t_{FER} b_3 + (b_1 + b_2 + b_3) \text{ for } t_{FER} < t \quad (4)$$

Where Y is the response variable (global yield, %); b_i (i = 0, 1, 2, 3) are the linear coefficients of lines; t is time (min); t_{CER} is the CER time (min); and t_{FER} is the FER time (min). Using the adjusted parameters, the global yield achieved in t_{CER} and t_{FER} were calculated using Eq. (4) (Y_{CER} and Y_{FER}). Next, Eq. (5) was used to calculate how much Y_{CER} and Y_{FER} represent in terms of global yield at 70 min, which was called recovery (R).

$$R(\%) = \left(\frac{Y_t}{Y_{70}}\right) \times 100 \quad (5)$$

Where: Y_t = response (global yield, %) at t_{CER} (min) or t_{FER} (min), as appropriate and Y_{70} = response (global yield, %) at 70 min.

2.8. Analytical methods

2.8.1. Total phenolic content (TPC), total flavonoids content (TFC) and chlorophyll (Chl)

Total phenolics content was determined by the Folin-Ciocalteu method, as described by Singleton et al. (1999). 0.5 mL of extract was mixed with 2.5 mL of Folin-Ciocalteu reagent (diluted 1:10 in distilled water). After 5 min, 2 mL of sodium carbonate (Na_2CO_3) solution at 7.5 % (w/v) was added. The mixture was then left to rest for 2 h in the absence of light, and the absorbance was read at a wavelength of 760 nm on a spectrophotometer (Kasuaki UV–VIS, Brazil). A standard curve of gallic acid was prepared ($R^2 = 0.9939$), and the results were expressed in mg of gallic acid equivalent (GAE) per g of sample.

Total flavonoids content was determined using the colorimetric method, based on the reaction with aluminum chloride ($AlCl_3$), as adapted by Arvouet-Grand et al. (1994). To 1 mL of the extract solution, 1 mL of 2 % aluminum trichloride ($AlCl_3$) in methanol was added. The solution was vortexed, stored in the dark for 30 min and the absorbance was measured at a wavelength of 415 nm. A standard curve of quercetin ($R^2 = 0.9917$) was prepared, and the results were expressed in mg of quercetin equivalent (QE) per gram of sample.

Chlorophylls were determined according to the methodology described by Porra et al. (1989). Absorbance readings were taken at three different wavelengths: 663, 646, and 750 nm for chlorophyll a, b, and a+b, respectively. The results were expressed in mg/g of sample, determined through Eqs. (6–8):

$$Chla = 12.25\left(A^{663} - A^{750}\right) - 2.55\left(A^{646} - A^{750}\right) \quad (6)$$

$$Chlb = 20.31\left(A^{646} - A^{750}\right) - 4.91\left(A^{663} - A^{750}\right) \quad (7)$$

$$Chla + b = 17.76\left(A^{646} - A^{750}\right) - 7.34\left(A^{663} - A^{750}\right) \quad (8)$$

Where Chl_a, Chl_b and Chl_{a+b} refer to chlorophyll a, chlorophyll *b* and chlorophyll a+b, respectively; and A^{663}, A^{646} and A^{750} refer to absorbance values at wavelengths 663 nm, 646 nm and 750 nm respectively.

2.8.2. Phenolic profile by HPLC-DAD-ESI-MS^n

Phenolic compounds separation and identification were conducted using the Agilent Series 1100 HPLC system equipped with a DAD diode detector and an electrospray ionization mass spectrometry system (ESI-MS^n) LC/MSD Trap VL, coupled to an Agilent Chem Station data processing station.

Fractions of extracts obtained by PLE were injected directly, without any prior treatment, with an injection volume of 20 µL, onto a Zorbax Eclipse XDB-C18 reversed-phase column (2.1 × 150 mm; 3.5 µm particle; Agilent, USA) at 16 °C. Solvents A (water/formic acid, 99.9:0.1, v/v) and B (methanol/formic acid, 99.9:0.1, v/v) were used (de Oliveira et al., 2023; Delalibera et al., 2024) at a rate of 0.19 mL/min (Pertuzatti et al., 2021). The solvent gradient employed was: 2 min, 95 % A and 5 % B; 25 min, 73 % A and 27 % B; 50 min, 25 % A, 75 % B; 55 min, 3 % A, 97 % B; 65 min, 3 % A, 97 % B; 75 min, 95 % A and 5 % B. The phenolic acids, flavonols and flavones were identified using their UV–Vis and MS/MS spectra.

Electrospray ionization tandem mass spectrometry (ESI-MS^n) was conducted under the following conditions for compound identification: negative ionization mode; dry gas (N_2) at 8 L/min; drying temperature of 350 °C; nebulizer at 40 psi; capillary at -3500 V; capillary exit offset at 70 V; skimmer 1, voltage at -20 V; skimmer 2, voltage at -60 V; 100 % compound stability; and scan range 100–1000 m/z.

2.8.3. Antioxidant capacity by DPPH and ABTS assay

The antioxidant capacity of the samples was determined by capturing the DPPH radical (2,2-diphenyl-1-picrylhydrazyl) using the method of Brand-Williams et al. (1995), with modifications. A 100 µL aliquot of the diluted sample extract was added to 3.9 mL of a 6×10^{-5} mol/L methanol DPPH solution, and the mixture was kept in the dark. A blank was prepared similarly, using the extraction solvent and sample solution without DPPH, while the negative control was prepared with DPPH and the extraction solvent, but without the sample. After 30 min of reaction, the absorbance was measured at 517 nm using a spectrophotometer (Kasuaki UV–VIS). A standard curve of Trolox ($R^2 = 0.9846$) was prepared and the results were expressed in µmol Trolox equivalents (TE)/g of sample.

The capture of the 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) radical (ABTS), the methodology described by *Re* et al. (1999) was employed. A 30 µL aliquot of the extract was added to 3 mL of the diluted ABTS solution. The mixture was incubated for 25 min at 30 °C in a water bath. After the incubation period, the absorbance was measured at 730 nm. The absorbance was read at a wavelength of 730 nm using a spectrophotometer (Kasuaki UV–VIS). To determine the antioxidant capacity, a standard curve of Trolox ($R^2 = 0.9901$) was prepared and the results were expressed in µmol TE/g sample.

2.9. Statistical analysis

The results were expressed as mean ± standard deviation. The Shapiro-Wilk's test was applied to verify the normality of the data. Subsequently, the experimental data were subjected to Analysis of Variance (ANOVA) to assess differences among the samples at a significance level of 5 %, using the Tukey's post hoc test. Pearson correlation analysis (ethanol and hydroalcoholic 50 %) and Spearman correlation were conducted. A p-value < 0.05 was considered the threshold for statistical significance. The software used was Statistica® 7.0 Software (Statsoft,

USA).

3. Results and discussion

Regarding the characterization of the vegetable matrix, moisture + volatile content was 7.61 ± 0.21 % by the gravimetric method and moisture content of 9.85 ± 0.59 % by the Karl Fischer method. Additionally, the sample used had an average particle diameter of 0.499 mm, apparent density of the bed was 0.29 ± 0.01 g/cm^3. This higher value found by the Karl Fischer method can be attributed to the high sensitivity characteristic of this method, while the oven drying method, in addition to not allowing complete water removal, as it is based on the loss of mass during the heating process, is not suitable for samples with low moisture content.

3.1. Global yield (X_0)

Table 1 presents the global extraction yield for PLE from *M. charantia* leaves using ethanol, hydroalcoholic 50 % (v/v) and distilled water as solvents under different temperatures and solvent flow conditions.

The values of X_0 ranged from 5.0 to 33.8 % and hydroalcoholic 50 % being the most efficient solvent in extractions. Among the analyzed parameters, flow rate was the only condition that showed no influence on the X_0 of PLE extracts from *M. charantia* leaves, according to ANOVA ($p < 0.05$). Conversely, the effect of temperature under different solvent conditions is noticeable, as its physicochemical properties (diffusivity, viscosity, surface tension, and dielectric constant) are altered, influencing the extraction process.

As previously reported hydroalcoholic mixtures are suitable solvents for obtaining high extraction yields in several leaves (de Souza et al., 2019; Maravić et al., 2022) and in fruits of *M. charantia* species under high pressure and temperature conditions (10 MPa, 50 to 150 °C) (Pitipanapong et al., 2007). De Aguiar et al. (2019) emphasize that the use of solvent mixtures promotes increased solubility and interaction of target compounds with the extraction solvents in a binary mixture. In this case, one solvent is responsible for the solubility of the analyte and the other solvent for the desorption of compounds bound to the plant matrix.

Furthermore, Sánchez-Camargo et al. (2016), when extracting algae by PLE, obtained X_0 values about three times higher in aqueous extracts compared to ethanolic ones, which may be related to the nature of the extracted components. These components generally refer to phenolic compounds bound to proteins or sugars, tannins, and some organic acids. The low X_0 results obtained using ethanol are related to the fact that this solvent is selective for compounds of moderate polarity (Viganó et al., 2016).

Table 1
Global yield (X_0, %) of *M. charantia* L. leaves extracts obtained by Soxhlet and PLE using different solvents flow conditions and temperature at fixed pressure of 12 MPa.

Flow rate	T (°C)	Global Yield (%)		
		Ethanol	Hydroalcoholic 50 %	Distilled water
1 g/min	**40**	6.6 ± 0.4^{b}	27.2 ± 2.4^{a,b}	16 ± 3^{c}
	50	7.3 ± 0.1^{b}	26.4 ± 3.2^{a,b}	18.4 ± 0.7^{b,c}
	60	8.5 ± 0.5^{b}	33.7 ± 2.8^{a}	20.5 ± 2.3^{b,c}
2 g/min	**40**	6.3 ± 0.6^{b}	22.2 ± 0.7^{b}	30.8 ± 2.6^{a}
	50	6.4 ± 1.3^{b}	31.2 ± 4.0^{a}	21.9 ± 2.5^{a,b,c}
	60	8.2 ± 1.0^{b}	31.5 ± 0.6^{a}	19.3 ± 0.5^{b,c}
3 g/min	**40**	5.0 ± 0.6^{b}	30.2 ± 1.6^{a,b}	19.1 ± 1.4^{b,c}
	50	6.4 ± 0.2^{b}	32.4 ± 0.1^{a}	23.2 ± 0.8^{a,b,c}
	60	8.7 ± 0.4^{b}	33.8 ± 0.5^{a}	27.6 ± 0.0^{a,b}
Soxhlet	**BT**	17.1 ± 2.7^{a}	-	24.4 ± 4.2^{a,b,c}

Values with different superscripts (a-g), in the same column, are significantly different ($p < 0.05$, Tukey's test). In the table, the results are expressed as a percentage ± standard deviation. BT: solvent boiling temperature.

The increase in extraction yield with temperature is noticeable, which is related to the well-established fact that higher temperatures can enhance compound solubility and solvent diffusivity while decreasing solvent viscosity. These aspects lead to better solvent penetration into leaf structures, increased mass transfer, breaking down lignocellulosic fibers and consequently, affecting the extraction yield (Fraguela-Meissimilly et al., 2024).

The Soxhlet extraction yield for ethanol solvent (17.1 %) was significantly higher than for PLE with the same solvent for all evaluated conditions (Table 1). In Soxhlet extractions, the solvent is employed at its boiling point, leading to decreased viscosity and surface tension. This alteration enables the solvent to effectively penetrate the solid matrix and dissolve the soluble substances. Furthermore, elevated temperatures promote solvent recycling and enhance interactions between the solvent and solute, contributing to efficient solubilization of components from the material (de Aguiar et al., 2014).

3.2. Total phenolic content (TPC) and total flavonoids content (TFC)

Table 2 presents the total phenolic content and total flavonoid content for PLE from *M. charantia* using ethanol, hydroalcoholic 50 % (v/v) and distilled water as solvents under different temperatures and solvent flow conditions.

The highest extraction yields of TPC were obtained with the 50 % hydroalcoholic solvent under the conditions of 50 °C and 3 g/min (14.5 mg GAE/g sample) and 60 °C and 2 g/min (14.4 mg GAE/g sample), except for one experimental condition using water (2 g/min and 60 °C).

These results indicate that the phenolic compounds present in *M. charantia* leaves exhibit high polarity. According to Mustafa and Turner (2011) the superior performance of the ethanol and water mixture is primarily due to the increased solubility and diffusivity of phenolics in this solvent at high temperatures, as well as the reduction in viscosity, surface tension, and disruption of Van der Waals, hydrogen, and dipole-dipole bonds, resulting in higher extraction yields. The 50 % hydroalcoholic solvent showed a high extraction yield of phenolics in most of studied extracts, consistent with findings reported by other authors when extracting phenolic compounds from plant matrices using PLE (de Aguiar et al., 2019; Viganó et al., 2016).

When analyzing the influence of temperature on TPC extraction yield, it exhibited a similar behavior to that observed for X_0 with the increase in temperature from 40 to 60 °C, increasing by up to 2.94 times for ethanol, 1.2 times for 50 % hydroalcoholic, and 2.1 times for water. Durmaz and Gökmen (2011) stated that cellular deformations can occur during PLE at elevated temperatures, thereby enhancing the extraction capacity of bioactive compounds and leading to the release or chemical modification of phenolic compounds from bound structures, such as lignin degradation.

Regarding the TFC of the PLE extracts, the results ranged from 1.8 to 10 mg QE/g sample, with the highest value found for extract obtained with ethanol (60 °C; 1 g/min) and the lowest value obtained with distilled water (40 °C; 1 g/min) as solvent. The TFC values presented in Table 2 suggest a reduction in the TFC content as the solvent polarity increases. This observation is consistent with the findings of Garmus et al. (2019), who reported the highest concentrations of TFC in the extracts obtained with ethanol and hydroalcoholic solvents using supercritical fractionation technology on *Eugenia uniflora* leaves.

According to Milião et al. (2022), UFP generally contains high amounts of phenolic compounds (bound, conjugated, or free), depending on the UFP species, tissue type, extraction method, and solvent. The TPC values for the extracts obtained by PLE in this study are similar to those reported by Torres et al. (2022) for the PLE process from the UFP *Pereskia aculeata*, Rodrigues et al. (2020) for the subcritical water extraction (SWE) process from the UFP *Cnidoscolus aconitifolius* (Mill.), and Kong et al. (2020) for the conventional solvent extraction from UFP *Bambusa vulgaris*.

For most of the evaluated PLE experimental conditions, an increase

Table 2
Total phenolic content (TPC) and total flavonoids content (TFC) of *M. charantia* L. leaves extracts obtained by Soxhlet and PLE under different solvent flow rates and temperatures at a fixed pressure of 12 MPa.

Flow rate	T (°C)	TPC (mg GAE/ g sample)			TFC (mg QE/ g sample)		
		Ethanol	Hydroalcoholic 50 %	Water	Ethanol	Hydroalcoholic 50 %	Water
1 g/min	**40**	1.9 ± 0.1^{g}	9.4 ± 0.2^{e}	4.4 ± 0.2^{h}	4.6 ± 0.1^{g}	4.3 ± 0.1^{g}	1.8 ± 0.0^{g}
	50	4.1 ± 0.1^{d}	10.5 ± 0.1^{d}	6.5 ± 0.1^{g}	9.4 ± 0.2^{c}	4.5 ± 0.0^{f}	3.2 ± 0.1^{c}
	60	5.6 ± 0.2^{b}	11.3 ± 0.1^{c}	8.1 ± 0.1^{f}	10 ± 0.1^{b}	5.3 ± 0.1^{d}	2.8 ± 0.0^{e}
2 g/min	**40**	2.6 ± 0.1^{e}	12.1 ± 0.1^{b}	10.5 ± 0.4^{b}	7.2 ± 0.1^{e}	5.2 ± 0.1^{d}	3.6 ± 0.1^{b}
	50	$4.6 \pm 0.5^{c,d}$	10.9 ± 0.1^{d}	$9.8 \pm 0.3^{c,d}$	$7.7 \pm 0.2^{d,e}$	4.8 ± 0.0^{e}	4.5 ± 0.2^{a}
	60	5.3 ± 0.3^{b}	14.4 ± 0.3^{a}	21.9 ± 0.1^{a}	8.1 ± 0.3^{d}	6.6 ± 0.1^{b}	2.3 ± 0.0^{f}
3 g/min	**40**	$2.3 \pm 0.2^{e,f}$	11.9 ± 0.1^{b}	6.5 ± 0.3^{g}	6.4 ± 0.1^{f}	5.8 ± 0.1^{c}	2.6 ± 0.0^{e}
	50	4.7 ± 0.2^{c}	14.5 ± 0.1^{a}	9.2 ± 0.2^{e}	7.5 ± 0.1^{e}	6.7 ± 0.1^{b}	2.8 ± 0.1^{e}
	60	5.8 ± 0.4^{b}	14.2 ± 0.2^{a}	$10.1 \pm 0.2^{b,c}$	$9.5 \pm 0.3^{b,c}$	7.1 ± 0.2^{a}	3.0 ± 0.0^{d}
Soxhlet	**BT**	8.6 ± 0.2^{a}	-	$9.5 \pm 0.2^{d,e}$	15.7 ± 0.5^{a}	-	2.8 ± 0.0^{e}

Values with different superscripts (a-j), in the same column, are significantly different ($p < 0.05$, Tukey's test). The results of TPC are expressed as mg GAE/g sample and TFC in mg QE/g sample. BT: solvent boiling temperature.

in the extraction yield of TFC can be observed with the increase in temperature at a fixed solvent flow rate regardless of the solvent used. This behavior may be related to the fact that temperature influences the dielectric constant and consequently, the polarity of solvents. When the temperature of water and ethanol is increased under constant pressure, their respective dielectric constants decrease, thus reducing their polarity as extraction solvents (Shang et al., 2014).

3.3. Determination of phenolic compounds by HPLC-DAD-ESI-MS^n

HPLC-DAD-ESI-MS^n was used to analyze individual phenolic compounds in *M. charantia* PLE extracts obtained with different solvents. It was possible to identify 24 phenolic compounds belonging to three classes: phenolic acids, flavones and flavonols. The profile of phenolic compounds varied depending on the PLE extraction solvent, as shown in Table 3 and Fig. 2.

When analyzing the phenolic compounds profile obtained with each extracting solvent, it can be observed that 16 compounds were identified in the aqueous extracts, the majority (10 compounds) belonging to the class of phenolic acids. These results corroborate the TPC and TFC analyses (Table 2), since it was observed that the aqueous extract has a higher concentration of TPC and a lower concentration of TFC than the ethanolic extracts.

Fewer phenolic compounds were found in the ethanolic extract than in the aqueous extract. Of the 12 compounds identified, 4 are phenolic acids, 4 flavones and 4 flavonols. The ethanol solvent stood out for its ability to extract flavonols, which were all derived from quercetin, namely: Quercetin 3 glucoside, Quercetin diglucoside, Quercetin arabinoglucoside and Quercetin 3-(6″-malonyl)-glucoside. Guarniz et al. (2019) comparing hydroalcoholic and acetone extracts of *M. charantia* leaves, observed that the hydroalcoholic extract managed to extract a quercetin-hexoside that was not identified in the acetone extract.

The 50 % hydroalcoholic solvent, due to the presence of water and ethanol, resulted in PLE extracts with an intermediate profile of phenolics, being able to extract more compounds when compared to ethanol and water solvents. A total of 20 compounds were identified, consisting of a mixture of phenolic acids, flavones, and flavonols observed in PLE extracts obtained with pure water and ethanol.

Table 3
Retention times, mass (negative ionization mode) of phenolic acids, flavones and flavonols in *Momordica charantia* extracts obtained by PLE.

Peak	Compounds	Pseudomolecular ion; product ions (m/z)	Water Rt (min)	Ethanol Rt (min)	Hydroalcoholic 50 % Rt (min)
	Phenolic acid				
1	Ferulic acid	193; 178	2.6	-	2.55
2	Caffeic acid hexoside	341; 179	-	2.956	2.949
3	Quinic acid	191; 173, 111	2.9	-	3.7
4	Vanilic acid	167; 123	19.7	-	
9	Dicaffeoylquinic acid	515; 335, 173	31.587		31.5
10	Protocatechuic acid hexoside	385; 223, 153	33.1	33.1	33.1
12	Caffeoylquinic acid	353;191, 173, 111	-	36.2	36.486
13	3-feruoyl-5-caffeoylquinic acid	529; 179	36.655	-	-
14	Feruoylquinic acid	368; 173, 111	40.646	-	40.678
15	Shikimic acid	173; 155, 129, 111	41,086	-	-
17	Tricaffeoylquinic acid	677; 335	42.612	-	42.614
20	Caffeoylshikimic acid derivative	692; 659, 335	44.478	44.468	44.465
	Flavone				
5	Luteolin 7-rutinoside	593; 447, 285, 151	24.018	23.9	23.9
6	Luteolin 7 glucoside	447; 285	25.978	25.5	25.3
7	Luteolin 7-arabinoside	417; 285,179, 151	29.233	30.2	30.2
8	Luteolin 7 galatoside	447; 285, 151	30.609	-	-
24	Luteolin 7-rutinoside	593; 447, 285, 151	-	47.479	47.485
	Flavonols				
11	Quercetin diglucoside	625; 463, 301	-	-	36
16	Quercetin diglucoside	625; 463, 301	41.438	41.4	41.524
18	Quercetin diglucoside	625; 463, 301	-	-	43.675
19	Quercetin arabinoglucoside	595; 301	-	44.098	44.094
21	Quercetin 3 glucoside	463; 301	-	45.280	45.274
22	Quercetin 3-(6″-malonyl)-glucoside	549; 505, 01	-	45.989	45.984
23	Quercetin diglucoside	625; 301	46.860	-	46.957

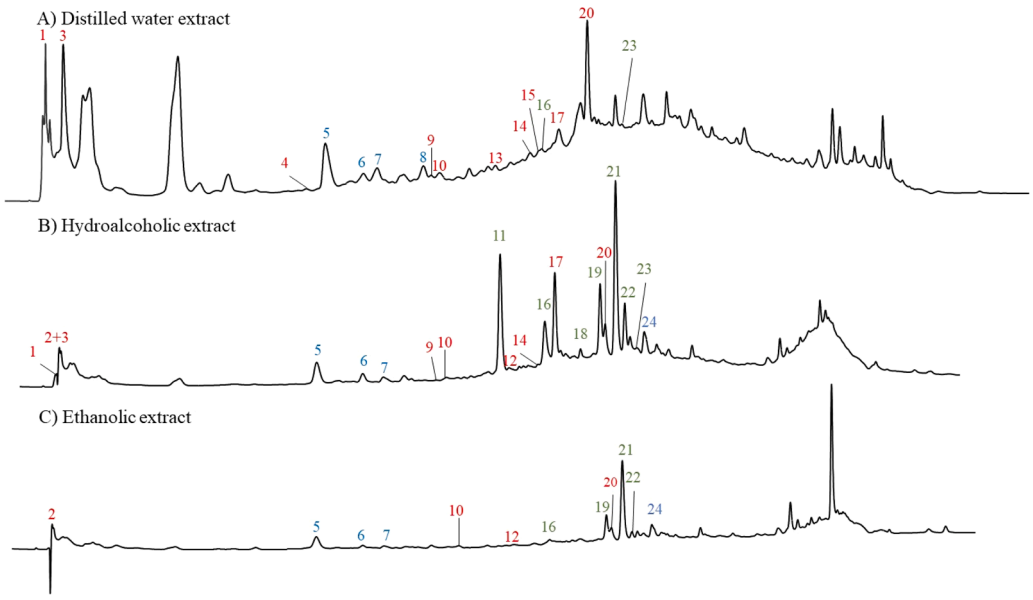


Fig. 2. HPLC-DAD chromatogram (detection at 280 nm) showing the phenolic profiling of *Momordica charantia* extracts. (A) Distilled water extracts, (B) Hydroalcoholic extracts, (C) ethanolic extracts. For peek assignment see Table 3.

3.4. Chlorophyll content (Chl)

The concentrations of chlorophyll in PLE extracts of *M. charantia* leaves are presented in Fig. 3. When ethanol was used as the solvent, the PLE process was able to recover the highest chlorophyll content under all extraction conditions evaluated, including the Soxhlet system. Chlorophyll content (mg/g sample) in PLE extracts ranged between 1.39 - 4.04, 0.46 - 1.75 and 1.85 - 5.79 for types a, b and a+b, respectively.

Water was the solvent that resulted in PLE extracts with lower amounts of chlorophyll. These compounds were not detected in the PLE water extracts obtained at a flow rate of 3 g/min (at temperatures of 40, 50, and 60 °C) and Soxhlet extract. A similar behavior was observed by Plaza et al. (2012) when extracting *C. vulgaris* by PLE with water at different temperatures (50, 100, 150, and 200 °C). According to Nguyen et al., (2023), chlorophylls reflects green light, and their degradation caused by temperature elevation converts the pigment into pheophytin, assuming an olive-yellow color, the predominant color observed in the aqueous extracts the present study.

Chlorophylls are present in the main primary groups of pigments and are responsible for converting carbon dioxide and water into organic compounds through light (Li et al., 2024). Chlorophylls a and b are the most abundant in nature and exhibit anti-inflammatory (Lee et al., 2017) and antimutagenic activities (Molina et al., 2022). Besides their applicability in the food industry, the cosmetics industry has also shown interest in this class of compounds, especially in the development of extraction processes employing green solvents, since they are commonly obtained using dimethylformamide, acetone, and ethyl ether solvents (Halim et al., 2010).

3.5. Antioxidant capacity

Fig. 4 presents the antioxidant capacity by the DPPH and ABTS radical scavenging methods of PLE and Soxhlet extracts from *M. charantia* L. leaves.

The values of antioxidant capacity using the DPPH method among the extracts obtained by PLE ranged from 10.72 to 218.96 μmol TE/g sample, with ethanol being the solvent that resulted in extracts with higher DPPH values. On the other hand, the extracts obtained by Soxhlet with ethanol showed a value up to 7 times lower than those obtained with the same solvent using the PLE method, corresponding to 30.85 μmol TE/g sample. The low antioxidant capacity of this extract may be related to the degradation of thermolabile compounds due to exposure to high temperatures for an extended period, characteristic of Soxhlet extraction. Additionally, as seen in Table 2, Soxhlet with ethanol solvent resulted in the highest extraction yield compared to PLE with the same solvent. High extraction yields lead to low selectivity, especially when using solvents like methanol and ethanol, resulting in the extraction of a wide range of compounds (de Aguiar et al., 2014), which can also lead to lower DPPH values in the extracts.

Part of the antioxidant capacity by the DPPH method is due to the presence of quercetin and its isomers in the PLE extracts of *M. charantia* leaves, as verified by the HPLC-DAD-ESI-MS^n analysis. According to Toth et al. (2018) in a DPPH analysis coupled with online HPLC for rapid screening of antioxidant phenolic compounds, quercetin exhibited the highest DPPH radical quenching activity, attributed to the presence of two free hydroxyl groups in the B ring.

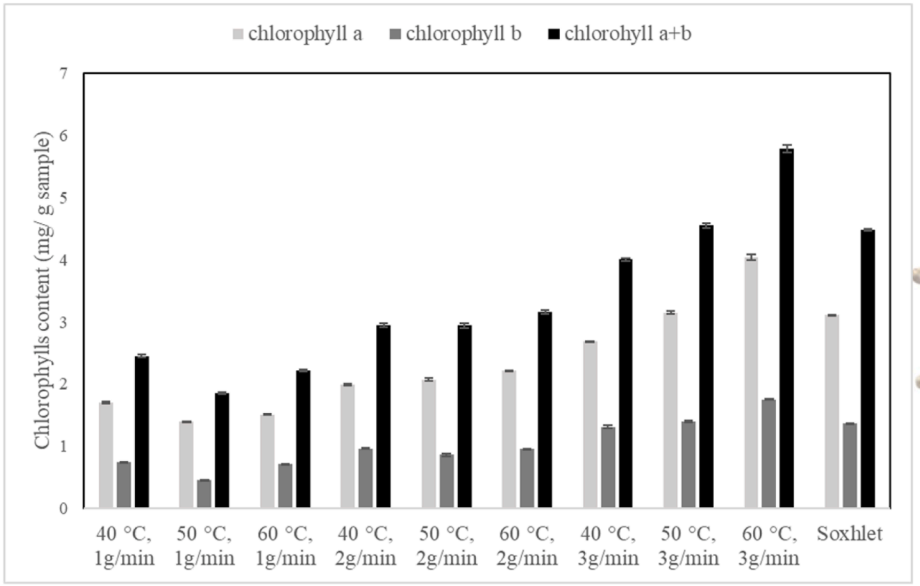
With regard to the antioxidant capacity of the extracts using the ABTS method, it can be observed from Fig. 4 that the solvent had a significant influence on the extraction process. Unlike what was observed for DPPH, for ABTS, the aqueous extracts showed the highest antioxidant capacity, while the ethanolic extracts showed the lowest antioxidant capacity. Budrat and Shotipruk (2009) reported that subcritical water extracts from *M. charantia* fruits exhibited high efficiency in capturing the ABTS radical. This is because the ABTS solution is more stable and suitable for determining water-soluble phenolic compounds (He et al., 2011). As highlighted in section "Determination of phenolic compounds by HPLC-DAD-ESI-MS^n", the aqueous extract contains phenolic acids, which are more soluble than quercetin derivatives flavonols, which are predominant in the ethanolic extracts.

3.6. Correlation analysis

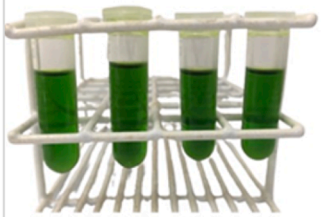
Fig. 5 presents the correlation analysis performed between the bioactive compounds and the antioxidant capacity of the extracts of *M. charantia* L. leaves in the three solvents studied.

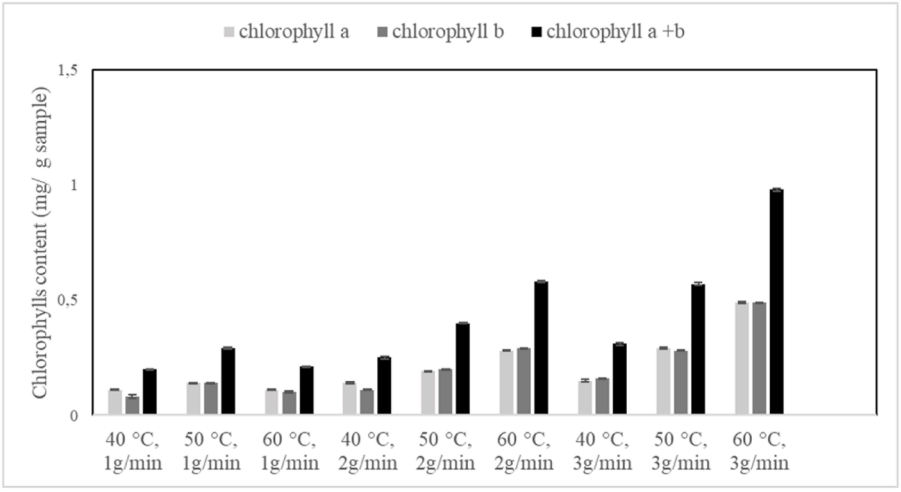
TPC showed high positive correlations with the antioxidant capacity in the three solvents used (ethanol, hydroalcoholic and water). The high degree of correlation between in vitro antioxidant capacity and total phenolic content has been well documented (Granato et al., 2018; Yang et al., 2024).

TFC did not present any correlation when the aqueous extract was evaluated, this is due to the low extraction capacity of flavonoids in



(A)





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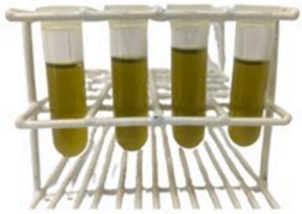

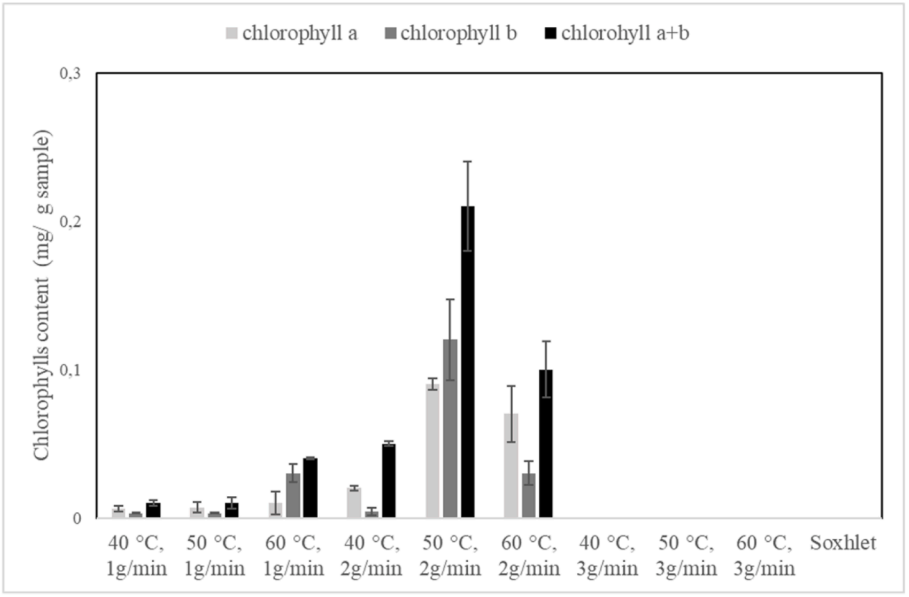

(C)

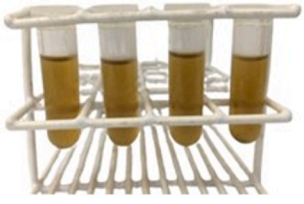

Fig. 3. Chl a, b and a+b of *Momordica charantia* L. leaf extracts obtained by PLE under different solvent flow rates and temperatures at a fixed pressure of 12 MPa and Soxhlet. (A) ethanol, (B) hydroalcoholic 50 % and (C) distilled water.

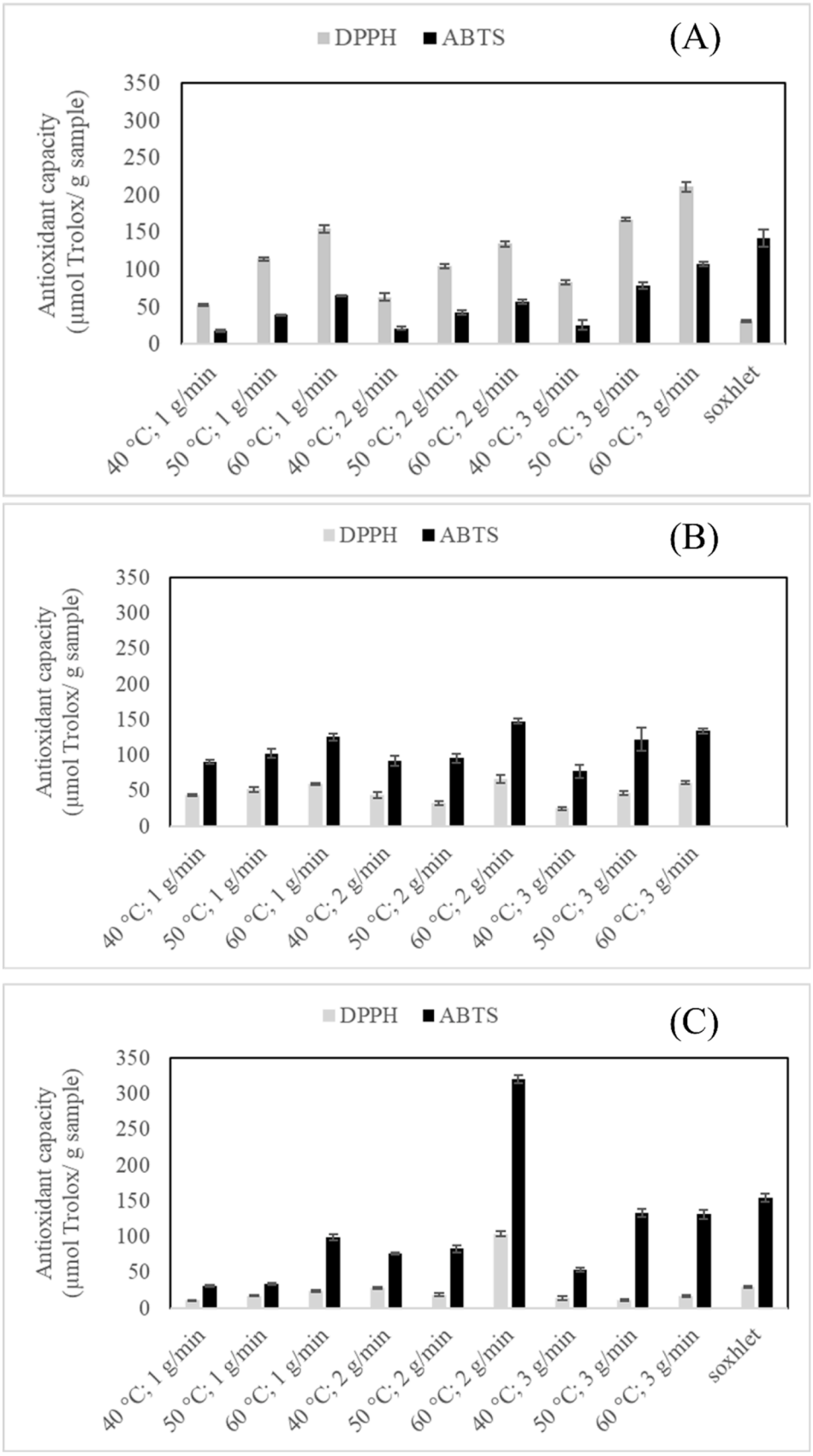

Fig. 4. Antioxidant capacity of *M. charantia* L. leaf extracts obtained by PLE under different solvent flow rates and temperatures, at a fixed pressure of 12 MPa and Soxhlet. (A) ethanol, (B) hydroalcoholic 50 % and (C) distilled water.

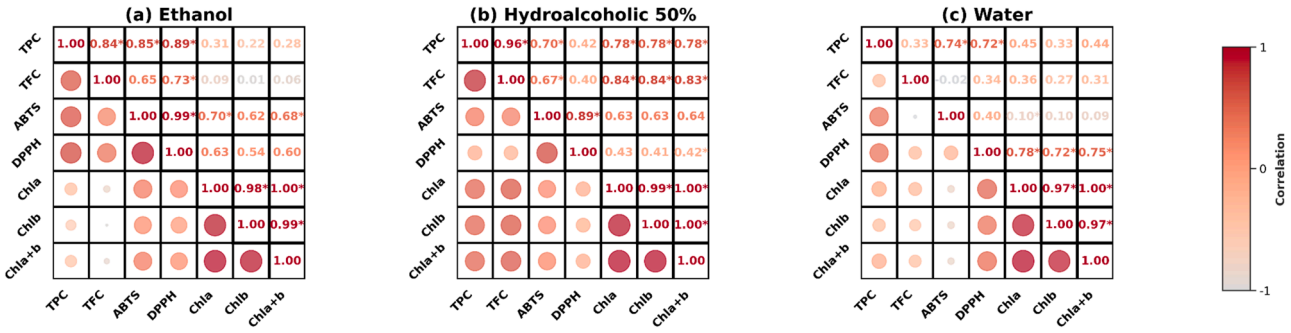

Fig. 5. Correlation analysis between bioactive compounds and antioxidant activity in vitro of the extracts of *M. charantia* L. leaves in the three solvents studied (* ≤0.05).

water discussed previously. However, in the extracts with high concentrations of flavonoids, strong positive correlations were observed, with the 50 % hydroalcoholic PLE extract showing nearly all significant correlations.

Another interesting finding from the correlation analysis relates to chlorophylls, which showed a strong positive correlation with TPC and TFC in the 50 % hydroalcoholic extract. It has already been shown that chlorophylls and some of their derivatives possess antioxidant capacity, as determined by the DPPH and ABTS methods, with their mechanism based on hydrogen atom donation in the absence of light (Alvarez-Parrilla et al., 2011).

3.7. PLE kinetics

The adjusted parameters by the three-line Spline model (t_{CER}, t_{FER}, R_{CER}, R_{FER}, Y_{CER}, Y_{FER} and R^2) applied to the PLE curves from *M. charantia* L. leaves for the 50 % hydroalcoholic solvent under different solvent flow conditions (1, 2 and 3 g/min) and temperature (40, 50 and 60 °C) are presented in Table 4. The 50 % hydroalcoholic solvent was selected as it yielded the best results in terms of extraction yield and extract composition. Fig. 6 shows the PLE kinetics experimental and adjusted by the three-line Spline model curves.

The adjusted extraction curves (Fig. 6) showed the three periods of an extraction process: the Constant Extraction Rate (CER) period, where solutes easily accessible to the solvent are predominantly extracted by convection; the Falling Extraction Rate (FER) period, where both mass transfer mechanisms, convection, and diffusion in the solid phase, control the mass transfer process; and the Diffusion Controlled (DC) period, in which mass transfer is controlled solely by diffusion, as described by Pereira and Meireles (2010).

Based on the determination coefficients (R^2) in Table 4, all curves were fitted with the three-line Spline model. It can also be observed that the CER time period varied significantly from 11.28 (60 °C, 2 g/min) to 13.19 min (60 °C, 3 g/min) considering all evaluated conditions. The majority of the global yield was obtained during the CER period (Y_{CER}) for all experimental conditions, ranging from 54.41 % to 73.93 % for the conditions of 40 °C, 1 g/min, and 60 °C, 2 g/min, respectively. According to Pereira and Meireles (2010), approximately 50 to 90 % of the total extract is obtained during the CER time. Furthermore, the authors state that the best operational conditions will be those in which high amounts of solute are extracted in a relatively short time, that is, during the CER period, highlighting the importance of this extraction stage in terms of scale-up and process design.

For the temperature of 40 °C, especially at a flow rate of 1 g/min, the R_{CER} value (54.41 %) indicates that the solvent did not perform well in extracting the material by convection. However, as the flow rate increases for the same temperature and solvent, an increase in the R_{CER} value is observed, reaching 68.96 % with a solvent flow rate of 3 g/min. Considering that the t_{CER} values had little variation among the studied conditions, it can be concluded that an increase in solvent flow rate implies an increase in the mass of solvent passing through the material to be extracted, which may result in an increased extraction yield due to the solute's solubility under process conditions.

The highest Y_{CER} (73.93 %) and Y_{FER} (94.73 %) values were obtained at a temperature of 60 °C with a flow rate of 2 g/min. The FER period allowed an increase in extract recovery of approximately 20 %. Thus, this experimental condition can be considered the most appropriate for scale-up in kinetic terms considering the global extract yield. The data regarding the extraction yield (31.5 %, Table 1), total phenolic content (14.4 mg GAE/g sample, Table 2), and total flavonoids content (6.6 mg QE/g sample, Table 2) suggest that the 50 % hydroalcoholic solvent condition, temperature of 60 °C, and flow rate of 2 g/min may also be the most suitable considering the extract composition in a large-scale PLE operation from *M. charantia* L leaves. However, to precisely define process conditions, studies on the kinetics of bioactive compounds extraction and process cost estimation are essential.

4. Conclusions

The findings of this study demonstrate that a 50 % hydroalcoholic

Table 4
Parameters adjusted by the three-line Spline model applied to the PLE curves from *Momordica charantia* L. leaf for the hydroalcoholic 50 % solvent under different solvent flow conditions (1, 2 and 3 g/min) and temperature (40, 50 and 60 °C).

Temperature (°C)	40			50			60		
Flow rate (g/min)	1	2	3	1	2	3	1	2	3
t_{CER}	12.10	12.09	11.82	11.80	12.35	11.95	11.88	13.19	11.28
Y_{CER}	14.82	15.29	20.83	15.79	21.96	19.79	21.81	23.29	23.43
R_{CER} (%)	54.41	68.73	68.96	59.80	70.35	61.14	64.67	73.93	69.36
t_{FER}	37.47	32.28	35.23	37.88	32.25	39.09	38.39	32.92	37.22
Y_{FER}	23.21	19.85	27.75	24.29	27.87	30.33	31.35	29.84	31.47
R_{FER} (%)	85.25	89.23	91.85	92.01	89.30	93.72	92.97	94.73	93.16
R^2	0.999	1	0.999	0.999	1	0.999	1	0.999	1

t_{CER}: CER time period; t_{FER}: FER time period; R_{CER} and R_{FER}: recovery at the end of t_{CER} e t_{FER}, respectively: Y_{CER} and Y_{FER}: extraction yield (%) at the end of t_{CER} e t_{FER}, respectively, and R^2: determination factor.

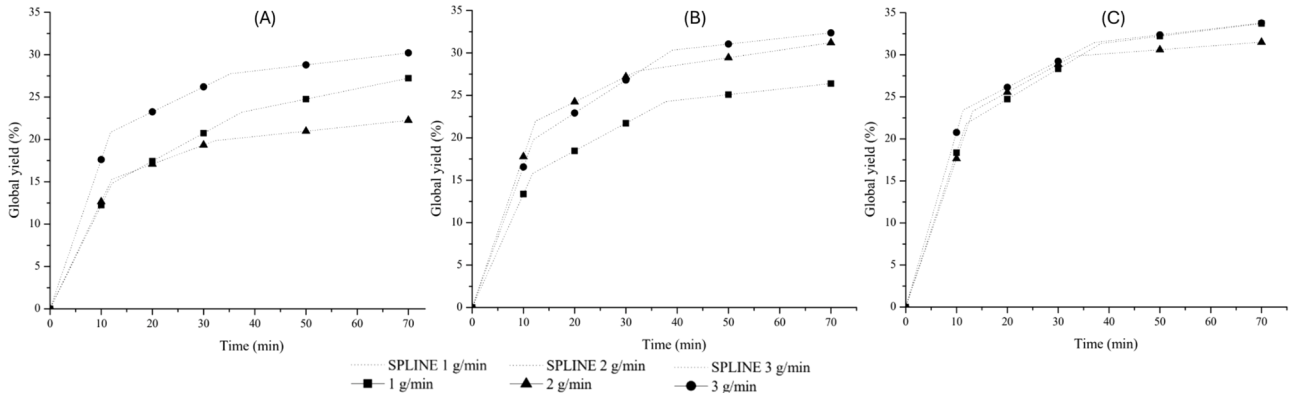

Fig. 6. PLE curves of global yield from *Momordica charantia* L. experimental and adjusted by the three-line Spline model for the hydroalcoholic 50 % solvent under different flow conditions (1, 2 and 3 g/min). (A) 40 °C, (B) 50 °C and (C) 60 °C.

mixture is the most efficient solvent for extracting bioactive compounds from *M. charantia* L. leaves by PLE. This solvent exhibited an intermediate phenolic compound profile compared to ethanolic and aqueous extracts. Chlorophyll content varied among the extracts, with the ethanolic PLE being more efficient in pigment extraction. In antioxidant capacity assays, ethanolic extracts showed higher DPPH capacity, while aqueous extracts exhibited greater ABTS radical scavenging capacity, likely due to differences in their chemical composition. The use of the Spline model enabled the identification of three distinct stages in the PLE extraction process. Optimal operational conditions, such as a temperature of 60 °C and a solvent flow rate of 2 g/min, were identified as effective for maximizing extraction during the CER stage. These results contribute to advancing extraction methodologies and highlight the potential of UFP in promoting sustainable food production, enhancing health benefits, and driving industrial innovation.

Ethical statement-studies in human and animals

The authors declare that this work has no studies in Humans and Animals.

CRediT authorship contribution statement

Gabriell J. R. Rocha: Writing – original draft, Validation, Methodology, Formal analysis. **Ana C. Aguiar:** Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Conceptualization. **Cátia O. G. Abud:** Methodology, Formal analysis. **Paula B. Pertuzatti:** Writing – original draft, Validation, Methodology, Investigation, Formal analysis. **Klicia A. Sampaio:** Supervision, Resources. **Julia T. Paula:** Writing – review & editing, Validation, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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Data availability

Data will be made available on request.

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