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Green extraction of phenolics from *Pouteria caimito* using microwave-assisted techniques

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ABSTRACT

Green solvents are being used to replace the conventional organic solvents in combination with green technologies. In this context, Peruvian *Pouteria caimito* fruit was used as a model for our study. The main objective was to explore an environmentally friendly strategy for the extraction of phenolics, by microwave or maceration using ethyl lactate and methanol as extraction solvents. Thus, each extract was compared by liquid chromatography coupled to tandem mass spectrometry (LC-ESI-MS/MS) to propose an alternative for the extraction processes and their antioxidant activity. The results showed that the two extraction methods had effects on the extraction yields, with microwave-assisted extraction (17.5% in MeOH and 8.0% in ethyl lactate) proving to be a better method for extracting the phenolic compounds than maceration extraction (12.2% in MeOH and 4.3% in ethyl lactate). In the case of LC/ESI/MS/MS studies the same trend was detected. Microwave extraction detected the presence of twenty-three compounds mainly including dicaffeoylquinic acid, monocaffeoyl quinic acids, feruloylquinic acids, galloylquinic acid, protocatechuic acid hexoside, ferulic acid and quercetin; while on maceration extraction between 15 up to 17 metabolites were identified. In this sense, microwave-assisted extraction was more efficient than maceration in terms of the number of metabolites extracted. The quantitative analysis of quercetin by high-performance liquid chromatography (HPLC) of all extracts showed no significant differences (around 8.704 up to 8.707 ± 0.019 mg/g extract). Finally, the antioxidant activity, in terms of ORAC (oxygen radical absorbance capacity) was higher in extracts prepared by microwave (93.51–97.05 mg TE/g extract) than in those prepared by maceration (77.25–86.88 mg TE/g extract). In the case of DPPH (2,2-diphenyl-1-picrylhydrazyl), the antioxidant activity was higher in extracts prepared by microwave (IC_{50}: 50.40–53.80 μg/mL) than in those prepared by maceration (IC_{50}: 67.6–74.10 μg/mL). Therefore, based on our results, a green extraction technique such as microwave-assisted extraction, compared to maceration, is faster, greener, and more sustainable alternative and could replace classical extraction techniques reducing negative side effects for both the environment and the analyst. These findings could be seen as a great potential for industrial and pharmaceutical processes to reduce the negative impact of using toxic organic solvents.

1. Introduction

The Pouteria genus is a pantropical group, which includes about 325 species, distributed throughout the Amazon region and Latin America. *Pouteria caimito* (Ruiz & Pav.) Radlk, known as “caimito or abiu”, with a probable origin in the Peruvian Amazon (Ribeiro de Souza et al., 2019), is a medium-sized tree, that can grow up to a height of 15 m (Aguilera et al., 2021). People in the Amazon region eat the pulp to relieve coughs, bronchitis and other lung diseases, while the latex is used as vermifuge and laxative (Vilela et al., 2016). Caimito fruits are consumed fresh or

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used in juice, liqueurs, ice cream, jellies, and desserts (Fortunato et al., 2021; Vilela et al., 2016).

Given the positive relationship between fruit intake and a lower risk of colorectal cancer, the antiproliferative and antioxidant activities were studied by Becker et al., 2020 on the human colorectal adenocarcinoma Caco-2 cell line, showing the potential of this fruit as an antioxidant with no cytotoxic effects (Becker et al., 2020). In this sense, Zhao et al., 2024 reported the positive health effects of consuming *P. caimito* fruits associated with their anti-inflammatory or antiviral properties, mainly linked to triterpenoids. This potential of *P. caimito* fruits opens up a new perspective for studying them using methodologies based on Green Chemistry applied to natural products, which can then be compared.

Based on Green Chemistry approaches, the 12 principles of this criterion have provided a framework for the design of both new chemical processes and the search of benign solvents. In the context of natural product chemistry, two of the twelve principles have a direct impact on solvents selection in separation processes or synthetic transformations: "wherever practicable, synthetic methods should be designed to use and generate substances with little or no toxicity to human health and environment" and "the use of auxiliary substances (e.g., solvents, separation agent, etc.) should be made unnecessary wherever possible, and also innocuous when being used" (Calvo-Flores et al., 2018; Whiteker, 2019). In this sense, a green solvent should present the following properties: obtained from renewable raw materials, competitive price with traditional solvent, recyclability, negligible toxicity, high biodegradability, properties and performances similar to common solvents, non-flammable and easy to transport. Having an ecological solvent should be associate with some ecological extraction methods known as non-conventional technologies (Majid et al., 2023). In the case of conventional extraction techniques, such as maceration, hydro-distillation, decoction, infusion, percolation, reflux and Soxhlet, they require large amounts of organic solvents, time, and involve high energy costs. In this line, there is a growing interest in the use of alternative technologies, which can reduce or eliminate the use of solvents harmful to the environment, with better yields, safety, and respect for the environment (Chemat et al., 2019; Majid et al., 2023). These green extraction methods include supercritical fluid extraction (SFC), pressurized liquid extraction (PLE), high pressure assisted extraction (HAE), electrically assisted extraction, microwave-assisted extraction (MAE) and ultrasound-assisted extraction (UAE) as the most common ones. These alternative techniques offer some advantages, such as lower consumption of organic solvents, shorter extraction times, less energy, and higher extraction yield of metabolites (Majid et al., 2023; Orio et al., 2012; Zhang et al., 2018).

In MAE, when electromagnetic waves are applied, polar molecules will continuously align according to the oscillating electromagnetic field. This dipolar rotation causes the rotating molecules to collide with adjacent molecules, transferring energy to them. Microwave heating can severely destroy the plant cell wall and release active compounds for higher extraction efficiency. In general, the advantages of MAE over conventional procedures include faster heating, lesser thermal degradation, and consumes less solvents providing a satisfactory extraction performance (Sing et al., 2022). MAE is considered an efficient green extraction technology for organic compounds such as caffeine, green tea polyphenols, essential oils from lilac buds, hesperidin from Citrus unshiu fruits, betalains from dragon fruits, phenolics from chokeberries, pectins from passion fruit, ginsenosides from ginseng root, silybinin and flavolignin from *Silybum marianum*, flavonoids and phenols from quince, and tannins (Soquetta et al., 2018; Sing et al., 2022; Majid et al., 2023; Jue & Nillianal, 2023).

A commonly used alternative is a combination of MAE and green solvents for environmentally friendly extraction of natural products. Green solvents are alternatives to conventional organic solvents due to their low toxicity and high biodegradability; they are recyclable, non-toxicity, non-volatility, low cost, available and sustainable produced from non-petroleum products. Some alternative solvents are ethyl lactate, cyrene, limonene, glycerol, deep eutectic solvents and water (Silva et al., 2018). In this context, six principles guide the green extraction of natural products: "1. Innovate by selecting varieties and using renewable plant resources. 2. Use alternative solvents, especially water or agro-solvents. 3.Reducing energy consumption through energy recovery and the use of innovative technologies. 4. Generation of by-products instead of waste including bio- and agro-refinery. 5. Reduction of operating units and promotion of safe, robust and controlled processes. 6. Aiming at obtaining non-denatured and biodegradable extracts without impurities" (Chemat et al., 2012). Ethyl lactate (ethyl-(*S*)-2-hydroxypropanoate) is obtained from naturally occurring raw material and can be used instead of toxic organic solvents in various phytochemical extraction processes. Some advantages of ethyl lactate are that it is biodegradable, easy to recycle, non-corrosive, non-carcinogenic, non-toxic, non-ozone depleting, has a low vapor pressure, a high boiling point, and low volatility. Ethyl lactate has demonstrated excellent solvating power in dissolving a wide range of organic molecules such as essential oils, pharmaceutical compounds, and natural products. Some applications are aimed at fragrances, inks coating factories, food additives, pharmaceutical preparations, organic synthesis, and as an extraction solvent for metabolites from natural sources (Shah et al., 2022; Usman et al., 2023).

Pouteria caimito is one of the tropical fruits found in the wild, with great potential for both exploitation and innovation strategies, though limited studies exist (Aguilera et al., 2021). Based on this information, the focus was on exploring an environmentally friendly strategy for the extraction of polyphenols from *Pouteria caimito* by MAE and maceration using ethyl lactate or methanol as extraction solvent. Finally, each non-conventional extract was compared by UHPLC-ESI-MS/MS to propose an environmentally friendly alternative in the extraction processes.

2. Materials and methods

2.1. Plant material

Fruits were collected in 2021 in "Distrito de Calleria", Pucallpa, Ucayali, Peru and identified as *Pouteria caimito* (Ruiz & Pav.). A voucher specimen (N° PCF-20211021) was deposited in the Herbarium of the "Museo de Historia Natural", Lima-Peru. Plant material was stored in sealed plastic bags in an ultra-freezer (Thermo TDE300) at −80 °C until use.

2.2. Chemicals

Ethyl lactate was purchased from Sigma Aldrich (Saint Louis, Mo, USA). Methanol (HPLC grade) and formic acid (for mass spectrometry, puriss. p.a.) were purchased from J.T. Baker (Phillipsburg, NJ, USA). Quercetin, gallic acid, dimethyl sulfoxide (DMSO), and trichloroacetic acid were purchased from Merck (Darmstadt, Germany).

2.3. Extraction by maceration

The powdered freeze-dried sample of *Pouteria caimito* (4.5 g) was added to 25 mL of methanol at room temperature (25 °C) with constant stirring (200 rpm) and extracted for 24 h. After centrifugation (9000 g, 30 min at 25 °C), the supernatant was concentrated in vacuum to yield 550 mg of a dark extract (12.2%). Under the same conditions, when ethyl lactate solvent was used, it yielded 193.5 mg (4.3%). Extractions were performed in triplicate following previous work (Calla-Quispe et al., 2020).

2.4. Microwave-assisted extraction (MAE)

The powdered samples of *P. caimito* (1.8 g) were placed in 10 mL of ethyl lactate. It was then placed in a microwave device (Anton Parr, Switzerland) with adjustable operating parameters (100 °C, 30 min, and

15 W). After centrifugation at 9000*g* for 30 min (25 °C), the supernatant was evaporated under vacuum to yield a gummy extract (144 mg; 8.0 %). Under the same conditions, when methanol solvent was used, it yielded 315 mg (17.5%). Extractions were performed in triplicate following previous work (Calla-Quispe et al., 2020).

2.5. UHPLC-ESI-HRMS/MS instrument

The tentative chemical identity of the target compounds was determined using a Dionex UltiMate 3000 HPLC system (Thermo Scientific, California, USA) connected to a high-resolution tandem mass spectrometry (HRMS/MS) detector with atmospheric pressure chemical ionization (APCI) source (Impact HD mass spectrometer Bruker, Massachusetts, USA) (HPLC-APCI-HRMS). For analysis, 2 mg of each extract was first dissolved in 2 mL of ethanol, then filtered (PTFE filter), and finally 10 μL was injected into the device, with all specifications set as previously described (Areche et al., 2020; Calla-Quispe et al., 2020).

2.5.1. HPLC parameters and MS parameters

Liquid chromatography was performed using a C18 column (Accucore, 150 mm × 4.6 mm ID, 2.5 μm, Thermo Fisher Scientific, Bremen, Germany) at 25 °C. Detection wavelengths were 254, 280, 330, and 354 nm, and PDA was recorded from 200 to 800 nm for peak characterization. The mobile phase was 0.1 % formic acid aqueous solution (A) and 0.1 % formic acid in acetonitrile (B). The gradient program (time (min), % B) was: (0.00, 12); (5.00, 12); (10.00, 20); (15.00, 40); (20.00, 40); (25.00, 70); (35.00, 12) and 15 min for column equilibration before each injection. The flow rate was 1.00 mL/min, and the injection volume was 10 μL. The standards and extracts dissolved in methanol were kept at 10 °C during storage in the autosampler. The parameters of the Impact HD mass spectrometer were set as previously described (Areche et al., 2020; Calla-Quispe et al., 2020).

2.6. HPLC quantitative analysis of quercetin

An HPLC-PDA equipment (Thermo Scientific Dionex Ultimate 3000 Series RS, Thermo Fisher Scientific, Germany) was used for the quantification of quercetin in *P. caimito* fruits. A 250 mm long 150 mm diameter 4.5 mm particle size, C18-RP column (Phenomenex, Torrance, CA, USA), in isocratic elution with a methanol-water-formic acid mobile phase (80: 15: 5 v/v) with a flow rate of 1.0 mL/min, a temperature of 27 °C, an injection volume of 20 μL, UV detection wavelengths of 254, 280, 320 and 550 nm, and an analysis time per sample of 30 min were used. The content of compounds in each prepared extracts was established with reference to the calibration curves of quercetin performed at 254 nm; and 15 min for column equilibration. A curve was performed with each of the individual standards (purity 98 %, by HPLC, from Biopurify, Chengdu, China). Measurements were performed in triplicate at each concentration level (5, 10, 20, 40, 60, 60, 80, 100 mg/mL) and with Chromeleon 7.2 software, the equation of the curve and the coefficient of determination were obtained (for quercetin $y = 0.0056x + 0.1339$, $R2 = 0.9989$; The results were expressed as mean ± standard deviation (SD).

2.7. DPPH test

This assay was performed using the DPPH decolorization method the according to Guerrero et al., 2019. Briefly, about 9 μL of each extracts (2 mg/mL) and 341 μL of methanolic DPPH solution (400 μM) were adjusted with MeOH to an absorbance of 1.10 ± 0.02 at 517 nm. The mixture was homogenized and allowed to react in the dark at room temperature (25 °C) for 20 min, after which the time absorbance at 517 nm was measured. The percentage of decoloration of the DPPH radical was determined by measuring the change in absorbance at 517 nm, and the values obtained were converted to percentage inhibition of the DPPH moiety. A curve was plotted with different dilutions of the extract, and the results were expressed as IC_{50} in μg/mL.

2.8. Oxygen radical absorbance capacity (ORAC) test

ORAC test was measured according to the method previously described by Guerrero et al., 2019. Fluorescein (3′,6′-dihydroxyspiro[2-benzofuran-3,9′-xanthene]-1-one) was prepared as a stock solution (4 μM) in 75 mM phosphate buffer pH 7.4, stored at 4 °C and used as a fluorescent probe. AAPH reagent (203.4 mg) was freshly prepared in 15 ml of 75 mM phosphate buffer, and used to generate the peroxyl radical. Trolox was used as an internal standard. In all experiments, 150 μl of fluorescein was added to each well. The blank wells received 25 μl of phosphate buffer, the standards received 25 μl of Trolox dilution, and the samples received 25 μl. Excitation was checked at 485 nm and emission at 528 nm with a bandpass of 20 nm on an HTX Multi-Mode Microplate Reader. Results were obtained by a quadratic regression equation (Trolox/sample vs. fluorescence decay curves), and expressed as mg Trolox/g extract.

2.9. Statistical analysis

Results were expressed as mean ± SD. The level of significance was set at $p < 0.05$. All statistical analyses were performed using GraphPad Prism 6 software for Windows.

3. Results and discussions

3.1. Extraction with conventional or non-conventional techniques

The powdered samples of *P. caimito* were extracted with MeOH and ethyl lactate (EL), using maceration (M) and microwave (MAE) techniques yielding four extracts. Extraction by maceration with methanol and ethyl lactate yielded 12.2% (M-MeOH), and 4.3% (M-EL) respectively. In the case of MAE, with methanol and ethyl lactate, it yielded 17.5% (MAE-MeOH), and 8.0% (MAE-EL) respectively. These results suggest that methanol as an extraction solvent, under both MAE and maceration conditions, is better than the ethyl lactate solvent.

3.2. Phytochemical profiling by UHPLC-ESI-HRMS/MS

The Phytochemical profile of the four extracts obtained by maceration and microwave, using ethyl lactate and methanol, are shown in Table 1. Detection wavelengths were set for the visualization of compounds with phenolic (280 and 330 nm) and flavonoid (254 and 354 nm) chromophores, and PDA was recorded for peak characterization. All extracts were compared and their metabolites were tentatively identified based on previously published LC/MS/MS data (Maldini et al., 2011; Abu-Reidah et al., 2013a,b; Iswaldi et al., 2013; Jimenez-Sanchez et al., 2015; Verardo et al., 2016; Guerrero-Castillo et al., 2019).

3.2.1. Amino acids and its derivatives

Peak 1 was tentatively identified as asparagine, with a molecular ion at *m/z* 131.0470. It was found in the four extracts (Guerrero-Castillo et al., 2019). Peak 5 was detected at R_T 5.25 min with an ion at *m/z* 290.0891 suggesting to be pyroglutamic acid hexoside (Abu-Reidah, Contreras, et al., 2013). This last compound was detected in all extracts except on M-EL.

3.2.2. Carbohydrates

Peak 2 and peak 3 were tentatively assigned as glucose and sucrose, with a [M-H] at *m/z* 179.0569 and 341.1082, respectively. Both compounds were detected in all extracts (Guerrero-Castillo et al., 2019).

3.2.3. Simple organic acids

Five compounds were identified as quinic acid (peak 4; $C_7H_{12}O_6$), citric acid (peak 6; $C_6H_7O_7$), succinic acid (peak 7; $C_4H_5O_4$), citramalic

Table 1
LC-ESI-MS/MS identification of metabolites from Peruvian *P. caimito* fruits.

Peak	T_R (min)	Compounds	Molecular formula	Theoretical mass	M-MeOH	M-EL	MAE-MeOH	MAE-EL	Experimental mass (MS/MS)	Mass error (ppm)
1	3.15	Asn	$C_4H_7O_3N_2^-$	131.0462	+	+	+	+	131.0470	6.1
2	3.29	Hexose	$C_6H_{11}O_6^-$	179.0559	+	+	+	+	179.0569	5.5
3	3.56	Sucrose	$C_{12}H_{21}O_{11}^-$	341.1089	+	+	+	+	341.1082 (165.0410)	2.1
4	3.82	Quinic acid	$C_7H_{11}O_6^-$	191.0561	+	+	+	+	191.0570 (127.0394)	4.7
5	5.25	Pyroglutamic acid-hexoside	$C_{11}H_{16}O_8N^-$	290.0881	+	–	+	+	290.0891 (128.0335)	3.4
6	5.39	Citric acid	$C_6H_7O_7^-$	191.0197	+	+	+	+	191.0201 (111.0087)	2.1
7	6.46	Succinic acid	$C_4H_5O_4^-$	117.0193	+	+	+	+	117.0189	3.4
8	7.37	Citramalic acid	$C_5H_7O_5^-$	147.0299	–	–	+	+	147.0307	5.4
9	10.73	Methyl citric acid	$C_7H_9O_7^-$	205.0354	–	–	+	+	205.0346 (111.0091)	3.9
10	13.40	Monocaffeoylquinic acid I	C_{16} $H_{17}O_9^-$	353.0899	+	+	+	+	353.0889 (191.0567; 179.0340)	2.8
11	13.79	Monocaffeoylquinic acid II	C_{16} $H_{17}O_9^-$	353.0899	+	+	+	+	353.0885(191.0565; 179.0343)	3.9
12	14.03	Monocaffeoylquinic acid III	C_{16} $H_{17}O_9^-$	353.0899	+	+	+	+	353.0883(191.0581; 179.0351)	4.5
13	14.57	Monocaffeoylquinic acid IV	C_{16} $H_{17}O_9$	353.0899	+	+	+	+	353.0887 (191.0569; 179.0355)	3.3
14	15.09	Feruloylquinic acid	$C_{17}H_{19}O_9^-$	367.1035	+	+	+	+	367.1030 (191.0560)	1.4
15	15.50	Feruloylquinic acid I	$C_{17}H_{19}O_9$	367.1035	+	+	+	+	367.1029 (191.0566)	1.6
16	16.57	Protocatechuic acid hexoside	$C_{13}H_{15}O_9^-$	315.0735	+	+	+	+	315.0730 (153.0199)	1.6
17	17.10	Dicaffeoylquinic acid	$C_{25}H_{23}O_{12}^-$	515.1183	+	+	+	+	515.1195 (353.0890; 191.0557; 179.0341)	2.3
18	18.44	Galloylquinic acid	$C_{14}H_{15}O_{10}$	343.0671	–	–	+	+	343.0662 (191.0571; 169.0153)	2.6
19	20.96	Ferulic acid	$C_{10}H_9O_4^-$	193.0506	+	–	+	+	193.0501	2.5
20	21.82	Quercetin	$C_{15}H_9O_6^-$	301.0309	+	+	+	+	301.0319	3.3
21	24.67	Tetrahydroxytricosanoic acid	$C_{23}H_{45}O_6^-$	417.3222	–	–	+	+	417.3212	2.4
22	25.47	dihydroxyheptadecatetraenoic acid	$C_{17}H_{25}O_5^-$	293.1758	–	–	+	+	293.1747	3.7
23	20.35	Dihydroxyoctadecanoic acid	$C_{18}H_{35}O_4^-$	315.2541	–	–	+	+	315.2538	0.9

acid (peak 8; $C_5H_7O_5$), and methylcitric acid (peak 9; $C_7H_{10}O_7$) (Gómez-Romero et al., 2010; Iswaldi et al., 2013; Abu-Reidah, Contreras, et al., 2013; Jimenez-Sanchez et al., 2015). Compounds 4, 6 and 7 were detected in all extracts, while compounds 8 and 9 were only detected in MAE-MeOH and MAE-EL, but not in maceration. Organic acids strongly influence the organoleptic properties of fruits, being responsible for their acidity (Abu-Reidah, Contreras, et al., 2013).

3.2.4. *Hydroxycinnamic acids*

Nine compounds were tentatively identified in this chemical group. Peaks 10–13 with the same molecular formula ($C_{16}H_{17}O_9$) were assigned to monocaffeoylquinic acids. They showed a [M-H] at *m/z* 353.0885 and a MS/MS ion at *m/z* 191.0570 corresponding to quinic acid. All monocaffeoylquinic acids were detected in all extracts (Abu-Reidah, Arraez-Roman, et al., 2013). Peaks 14 and 15 were assigned as feruloylquinic acids based on [M-H] and MS/MS (*m/z* 367.1030 and *m/z* 191.0570) (Verardo et al., 2016; Abu-Reidah, Arraez-Roman, et al., 2013). Peaks 17, 18 and 19 were identified as dicaffeoylquinic acid, galloylquinic acid, and ferulic acid, based on [M-H] and MS/MS data respectively (Maldini et al., 2011; Verardo et al., 2016). Peak 18 was found in both MAE-MeOH and MAE-EL, but not in maceration extracts. Ferulic acid was detected in both M-MeOH, MAE-EL and MAE-MeOH extracts, but it was not extracted by M-EL.

3.2.5. *Flavonoids*

Peak 20 was tentatively assigned as quercetin, which was detected in all extracts.

3.2.6. *Hydroxybenzoic acid*

Peak 16 was assigned by HRMS and MS/MS as protocatechuic acid hexoside (Abu-Reidah, Arraez-Roman, et al., 2013; Guerrero-Castillo et al., 2019). This compound was found in all extracts.

3.2.7. *Lipid derivatives*

Three compounds of lipid nature were tentatively detected from *P. caimito,* including tetrahydroxytricosanoic acid (peak 21), dihydroxyheptadecatetraenoic acid (peak 22), and dihydroxyoctadecanoic acid (peak 23) (Guerrero-Castillo et al., 2019). These lipids were only identified in the MAE extracts, but not in maceration, using solvents as MeOH and ethyl lactate. The following Fig. 1 illustrates structures of some of the compounds detected as an example.

In this work, a comparison was made between conventional (maceration) and non-conventional (microwave) techniques for the extraction of secondary metabolites from *P. caimito* fruits, using methanol (toxic solvent) and ethyl lactate (green solvent). Alternative solvents along with the microwave techniques were selected considering their cost, safety and availability. In our case, MAE showed better extraction of metabolites than maceration according to LC-ESI/MS/MS data. Regarding the extraction performance, our findings showed that methanol has better extraction efficiency, which could be due to high dielectric constant of methanol to solubilize the presence of mucilage from the pulp (Vilela et al., 2016). Peak 19 (ferulic acid) and peak 5 (pyroglutamic acid hexoside) were not found in M-EL, which could be due to lower polarity than methanol (Sepulveda et al., 2023). A similar work compared three solvents (methanol, ethanol and EL) in extracting of flavonoids from *Veronicastrum latifolium.* Ethyl lactate was the best extracting agent, showing stronger interaction and affinity for flavonoids (Yin et al., 2019). MAE compared to maceration as an extraction method was more efficient in the number of metabolites extracted (Table 1). In MAE, factors such as microwave power and treatment time are important due to potential risk of chemical degradation. Considering these factors, MAE is a greener, safer, and simpler technique than maceration (Chandrasekar et al., 2015; Zhan et al., 2018). Peaks 8, 9, 18, 21–23 were extracted by using microwave, but not in maceration. Microwaves produce heat, which results in a higher release of metabolites into the organic solutions. In our case, the microwave power was at 15 W (100 °C, 30 min) according to previous optimization (Calla-Quispe et al., 2020). In our case, heat had a positive effect on the amount of metabolites extracted. Similar results were reported on the extraction of phenolics from *Gordonia axillaris* fruits. In these sense, the extracts

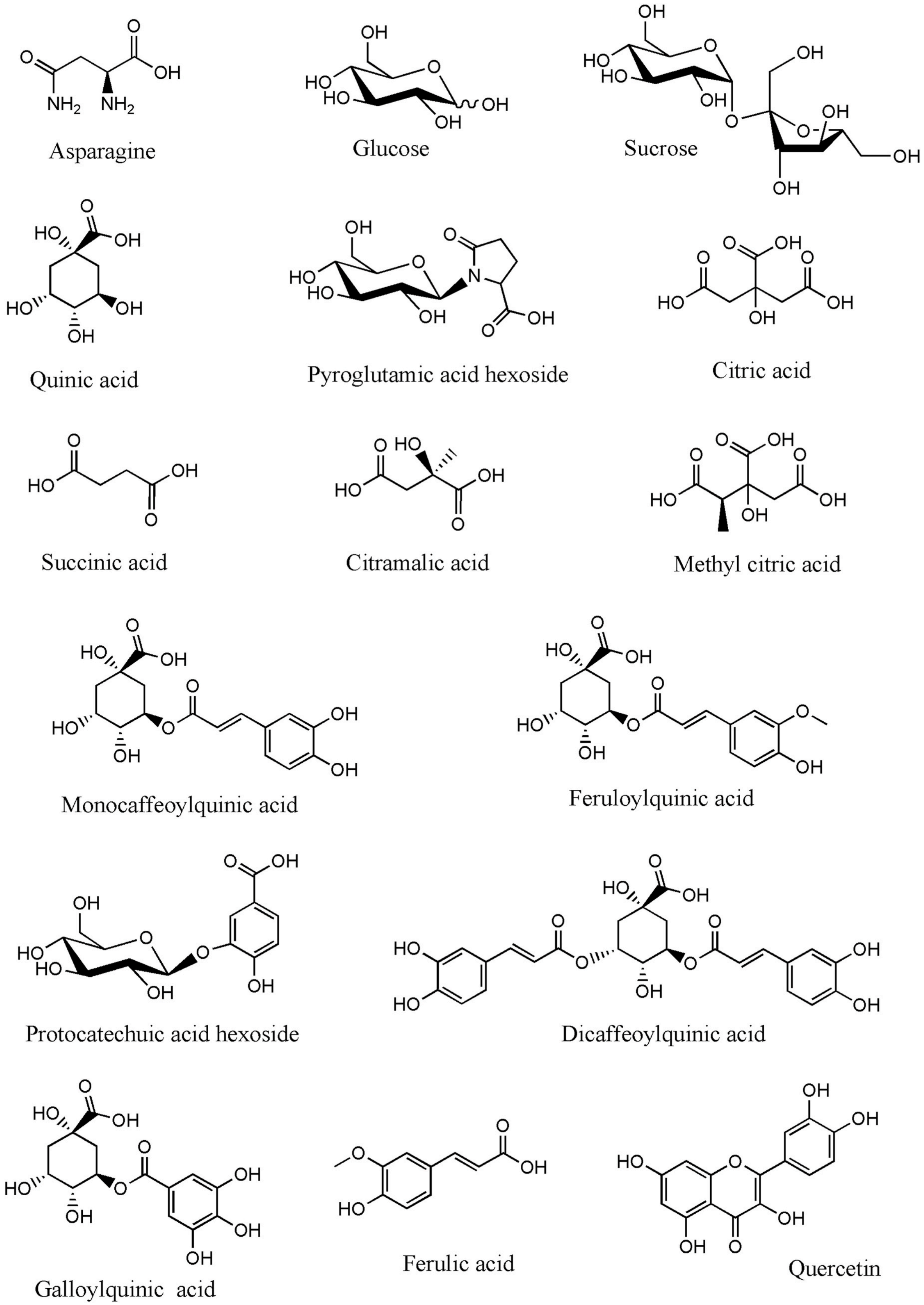

Fig. 1. The main compounds identified by LC/ESI/MS/MS from *P. caimito* fruits.

obtained by MAE showed higher phenolic content based on TEAC, TPC, and TFC, than those obtained using conventional extraction methods, including Soxhlet and maceration (Li et al., 2017).

Twenty-three metabolites have been tentatively identified from the fruit of *Pouteria caimito*, including amino acids, carbohydrates, organic acids, flavonols, hydroxycinnamic acid derivatives, hydroxybenzoic derivatives, and lipid compounds. A study based on UPLC-QTOF-MS analysis of an ethyl acetate extract of *Pouteria caimito* leaves detected the presence of eight triterpenoids known as oleanolic acid, ursolic acid, betulinic acid, euscaphic acid, heptadienic acid, 3*β*-*O*-(*p*-coumaroyl)-alphitolic acid, 3*β*-*O*-(*p*-coumaroyl)-maslinic acid and 3*β*-*O*-(*p*-coumaroyl)-2-hydroxy-12-ursen-28-oic acid (Ribeiro de Souza et al., 2019). In our study, we did not detect the presence of these triterpenoids in *P. caimito* fruits under the conditions used. Another study by Becker et al., 2020 reported the identification of eighteen phenolic compounds by UHPLC-OT-HRMS from *P. caimito* fruits from a sample from the Brazilian Amazon, including quinic acid, caffeoylquinic acid, and ferulic acid. (Becker et al., 2020).

According to safety square defined by Standard System for the Identification of the Hazards of Materials for Emergency Response (NFPA 704) is necessary to declare the risk associated to methanol and ethyl lactate solvents (see safety data sheet provided by Sigma-Aldrich). The red code indicates flammability; in the case of methanol, it is level 3, while ethyl lactate is level 2, indicating that ethyl lactate presents a lower fire hazard than methanol. The blue code indicates the level of health hazard, methanol is at level 1, while ethyl lactate is at level 3, indicating that ethyl lactate poses a higher health hazard than methanol (should only be used under a fume hood). The yellow code indicates chemical reactivity. In the case of both solvents, they are classified as level 0, indicating chemical stability. The white code indicates specific hazards. In relation to acute toxicological effects, methanol displays 100.1 mg/kg per oral, while ethyl lactate displays $LD_{50} > 2000$ mg/kg. Finally, methanol is a high flammable liquid, toxic if swallowed or inhaled, and can cause damage to organs, while ethyl lactate is a flammable liquid that can cause respiratory irritation and eye damage. Based on this information, it is important to demonstrate the application of green solvents that can replace toxic organic solvents in the process industry. The higher application of green solvents will reduce the use of hazardous conventional solvents and make the Earth greener (Shah et al., 2022; Usman et al., 2023).

3.3. Antioxidant activity

The antioxidant activity was determined by ORAC and DPPH (Table 2). Extraction by maceration using methanol as the solvent (M-MeOH) showed better capacity than M-EL in terms of both ORAC and DPPH antioxidant capacities (86.88 mg Trolox/g extract and 67.60 μg/mL). In the case of extraction by microwave using methanol (MAE-MeOH) or ethyl lactate (MAE-EL), no significant differences were observed based on ORAC assay and DPPH test (Table 2). When comparing all extracts, in terms of ORAC, the best samples were MAE-MeOH and MAE-EL (no significant differences were detected in both techniques associated with their solvents). The same trend was observed in the case of DPPH test. These findings could be attributed to the efficiency of MAE as solubility, and mass transfer associated with extraction time, power, and temperature were improved (Da Silva et al., 2022). Our results regarding *Pouteria lucuma* and other Chilean fruits were lower when compared (INTA, 2024 accessed on 12-27-2024). Fruit pulps contain generally compounds with functional potential, such as carotenoids, vitamins, steroids, flavonoids, and fibers. In the case of our extracts from *P. caimito*, they showed several phenolic compounds that can be regarded as antioxidants and green extracts can be possible candidates to use in industry. Green extracts from fruits can be used in various industries, including food and beverages, cosmetics, pharmaceuticals, and textiles (Soquetta et al., 2018). These extracts are generally composed of the same natural bioactive compounds found in fruits, and they offer several potential benefits due to their antioxidant, antimicrobial, and anti-inflammatory properties (Palos-Hernández et al., 2022). As an example, *Pouteria lucuma* NADES green extracts were composed of epigallocatechin isomers with antioxidant activities (Puma-Isuiza et al., 2024). A sample from *P. caimito* growing in Brazil was reported to have 3.11 mg ascorbic acid/100 g fresh weight, and 2.89 μmol Trolox/100 g fresh weight, (Seixas et al., 2021). Our extract ORAC values were higher than those reported for mangoes (18.22 μmol TE/g dry weight) but lower than those for medlar (192.79 μmol TE/g dry weight), maqui berries (371 μmol TE/g dry weight), and murta *Ugni molinae* berries (222.78 μmol TE/g dry weight) (INTA, 2024). The Bleaching of ABTS radical was lower than that found in the superfruits antioxidant Chilean blueberries and murta berries (IC_{50}: 3.32 and 10.94 and μg/mL, respectively) (Ramirez et al., 2015), It is important to state that if an extract or compound has antioxidant activity in vitro it does not mean that it will have the same capacity in vivo. The antioxidant capacities showed make these techniques adequate for the possible use of *P. caimito* green extracts in the industry.

Table 2
ORAC and DPPH capacity of all extracts obtained from *P. caimito* fruits.

Sample	ORAC*	DPPH*
M-MeOH	86.88 ± 2.17	67.60 ± 5.6
M-EL	77.25 ± 3.5	74.10 ± 4.9
MAE-MeOH	97.05 ± 1.68^{a}	53.80 ± 3.9^{b}
MAE-EL	93.51 ± 2.88^{a}	50.40 ± 3.5^{b}
Ascorbic acid	–	5.80 ± 1.2

All values are expressed as means ± SD (n = 5). *micrograms per gram of plant. Values in the same column marked with the same letter are not statistically different ($p < 0.05$). ORAC (mg Trolox/g extract) and DPPH (IC_{50} in μg/mL).

Regarding the presence of secondary metabolites and biological activities of *P. caimito*, Sanchez-Capa et al., 2023 reported an excellent review of edible fruits from the Ecuadorian Amazon, including *P. caimito* among them. The same study describes that *P. caimito* has antioxidant properties including DPPH, and FRAP, as well as content of ascorbic acid, polyphenolics, and carotenoids (Sanchez-Capa et al., 2023). Another study reported the quantification of phenolic compounds as caffeic acid and caffeoylquinic acids in sixty-four fruits consumed by people in Brazil. It determined high quantities of 3-caffeoylquinic acid in *P. caimito* (0.8–21.32 mg/kg), with significant variation among the samples obtained by different suppliers. In this same study, the presence of caffeic acid was not detected (Dillenburg et al., 2019). According to our results, the quantitative analysis of the concentration of quercetin was the same in all extracts showing no significant differences (8.707 ± 0.019 mg/g extract for maceration and 8.704 ± 0.005 mg/g extract for MAE; data not shown). This implies that increasing temperature does not have effects in the extraction of quercetin. The best combination of technique and solvents was microwave with ethyl lactate, representing an alternative to the conventional chemistry of natural product. Therefore, the use of non-conventional techniques combined with green solvents can open new paths for the extraction of compounds in different matrices.

4. Conclusions

Conventional techniques such as maceration with toxic solvents have proven to be harmful to both human beings and the environment. In this context, this research presents a comparative study on the extraction techniques of metabolites from *Pouteria caimito* fruit using two different techniques (maceration and microwave) in combination with methanol (toxic solvent) or ethyl lactate (green solvent). The results showed that the two extraction methods have significant effects on extraction yields, with MAE (17.5% in MeOH and 8.0% in EL) proving to be a better method for extracting phenolic compounds than maceration extraction (12.2% in MeOH and 4.3% in EL). The same trend was found in the metabolomic profile by LC/ESI/MS/MS. The microwave extraction detected the presence of twenty-three compounds, while on maceration extraction between 14 and 16 metabolites were tentatively identified. The quantitative analysis of quercetin by HPLC of all extracts showed no significant differences. Finally, the antioxidant activity in terms of ORAC and DPPH was higher in the microwave extracts. Therefore, based on our findings, a combination between a non-conventional technique, with the use of ethyl lactate as green solvent, could replace classic solvents and provide more data to use alternatives based on green chemistry. Technology is advancing rapidly towards new automated, more environmentally friendly methods for obtaining extracts and purifying natural compounds. Natural product chemists have been encouraged to use modern extraction technologies, as some of those green processes are expected become routine in both research laboratories and industries due to safety, environmental, and regulatory affairs.

CRediT authorship contribution statement

Jean Paul Miranda: Writing – original draft, Methodology, Data curation. **Nicolas Cifuentes:** Writing – original draft, Methodology, Formal analysis. **Daniela Bárcenas-Pérez:** Writing – original draft, Methodology. **Jan Hájek:** Writing – original draft, Software, Methodology. **José Cheel:** Writing – review & editing, Methodology. **Mario J. Simirgiotis:** Writing – review & editing, Methodology. **Beatriz Sepúlveda:** Writing – original draft, Methodology, Funding acquisition. **Carlos Areche:** Writing – review & editing, Writing – original draft, Funding acquisition, Conceptualization.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fbio.2025.106182.

Data availability

The data originate of this study are contained within the article.

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