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A sustainable approach to obtain polyphenols from Chilean wild murta, *Ugni candollei* B., and *Ugni molinae* T., using eutectic solvents and advanced extraction techniques

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ABSTRACT

Murta, native to southern Chile, comprises red murta (*Ugni molinae* Turcz) and white murta (*Ugni candollei* Barm), traditionally utilized in ethnobotanical medicine for its anti-inflammatory and analgesic properties attributed to high flavonoid and phenolic acid content. Despite murta's potential, the combined effects of sustainable extraction techniques—eutectic solvents (ES), microwave-assisted extraction (MAE), and ultrasound-assisted extraction (UAE)—on its polyphenol profile remain unexplored. A comprehensive analysis should quantify extractable polyphenols (EPP) and non-extractable polyphenols (NEPP). This study evaluated eight ES mixtures for polyphenol extraction from red and white murta leaves and fruits, optimizing water percentage and feed:solvent ratio. Choline chloride:1,3-butanediol (ChCl:1,3BD) with 30 % water and a 1:10 ratio yielded the highest EPP content, as determined by HPLC-DAD. Among various MAE and UAE conditions tested, MAE at 353 K for 3 min achieved optimal phenolic compound yields, with catechin predominating in leaf extracts and gallic acid in fruit extracts. NEPP fractions, consisting primarily of non-extractable proanthocyanidins, represented 8–19 % of total polyphenols in fruits and leaves. These findings establish a sustainable methodology for obtaining polyphenol-rich extracts from murta and highlight the importance of both EPP and NEPP fractions in enhancing the potential of these antioxidant-enriched food extracts obtained through eco-friendly technologies.

1. Introduction

Fruits and vegetables are rich in phenolic compounds; these are secondary metabolites highly valuable due to their biological properties (Rodríguez-Mateos et al., 2014). Edible wild berries are particularly known for their high phenolic compound content, and many studies have found that these fruits or their extracts can modulate several metabolic processes and benefit human health (Wan et al., 2023). The South of Chile is home to a variety of endemic berries that are characterized by their high content and diversity of polyphenols with high antioxidant capacity; this comprises fruits like arrayán (*Luma apiculata*) (Millán et al., 2023), maqui (*Aristotelia chilensis*) and murta (*Ugni molinae* Turcz and *Ugni candollei* Barm) (Rivera-Tovar et al., 2018). The latter is a shrub that grows up to 2 m high and belongs to the *Myrtaceae* family. This plant has significant cultural and economic importance, with a growing market for the fruits and traditional medicinal uses for the fruits and leaves (Vega-Galvez et al., 2020). In addition, in Chile, traditional medicine has employed the infusion of murta leaves and branches to address urinary tract infections and diabetes, and research has indicated that murta possesses anti-inflammatory properties

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(Rubilar et al., 2006).

The interest in murta is mostly derived from its high phytochemical content. Thus, bioactive compounds found in murta mainly include phenolic acids (gallic acid, caffeic acid-3-glucoside, ferulic acid, and *p*-coumaric acid), anthocyanins (several derivatives from cyanidin, delphinidin, malvidin, pelargonidin, peonidin and petunidin), other flavonoids (quercetin-3-*O*-glucoside, kaempferol, kaempferol-3-*O*-glucoside, myricetin, rutin, quercitrin, luteolin, or luteolin-3-glucoside) and triterpenic acids (Aguirre et al., 2006; Arancibia-Radich et al., 2016; Goity et al., 2013). While most studies on murta's polyphenolic profile have focused on *Ugni molinae* Turcz (red murta), some have investigated *Ugni candollei* Barm (white murta) (Avello-Lorca et al., 2016; Fuentes-Jorquera et al., 2024). Notably, more than 100 phenolic compounds were recently identified in white murta. However, it should be remarked that the vast majority of these studies are focused on the so-called extractable polyphenols (EPP), *i.e.*, polyphenols that may be released from the food matrix with a specific combination of solvents, but only a few have studied the fraction of non-extractable polyphenols (NEPPs) (López et al., 2017; Ospina-Posada et al., 2024). NEPPs are either polymeric polyphenols or single polyphenols linked to macromolecular food constituents, and they are not extracted by common aqueous-organic procedures (Pérez-Jiménez & Saura-Calixto, 2015). NEPPs are divided into two categories: hydrolyzable polyphenols (HP), which are low molecular weight phenolic compounds strongly associated with polysaccharides or proteins, and non-extractable proanthocyanidins (NEPA), which are high molecular weight structures. NEPPs have been shown to contribute significantly to the total polyphenol content and biological activities of plant foods (González-Sarrías et al., 2017; Pérez-Jiménez & Saura-Calixto, 2018). Thus, a comprehensive analysis of murta polyphenols should include the assessment of the NEPPs fraction.

Most previous studies on murta polyphenols have applied conventional extraction methods using organic solvents, such as ethanol, methanol, and water (Junqueira-Gonçalves et al., 2015; Shene et al., 2009). However, sustainable extraction processes such as microwave-assisted extraction (MAE) and ultrasound-assisted extraction (UAE) are efficient for recovering polyphenols from various sources, offering environmental and economic benefits (Ameer et al., 2017; López-Salas et al., 2024). Similarly, there is a growing interest in the use of eutectic solvents (ES), *i.e.,* mixtures of hydrogen bond acceptors (HBA) and hydrogen bond donors (HBD), as alternatives to conventional organic solvents for polyphenol extraction. ES offer several advantages, including low vapor pressure, non-flammability, and low toxicity. They are also more stable and biodegradable than traditional solvents (Tang et al., 2023; Zainal-Abidin et al., 2017). In particular, some of these techniques have been applied to berries. For example, UAE with several ES was used to extract polyphenols from leaves and branches of arrayán *(Luma apiculata*), finding that choline chloride:oxalic acid provided an extraction yield 2-fold higher than that obtained with the control solvent (Water/EtOH 80 % *w/w*) (Millán et al., 2023). Additionally, a ternary eutectic solvent composed of choline chloride, betaine hydrochloride, and levulinic acid achieved a polyphenol extraction yield 3.26 times higher than the control solvent (Water/EtOH 80 % *w/w* 75 % *w/w*) when used on cranberry pomace (Alrugaibah et al., 2021). However, applying these novel and more sustainable approaches to extract polyphenols from murta remains largely underexplored. A previous study with white murta recently compared ES and hot pressurized water extraction as two alternative techniques for obtaining EPP-rich fractions from murta (Fuentes-Jorquera et al., 2024). However, the impact of combining MAE and UAE with ES on total polyphenols in murta, *i.e.*, assessing both EPP and NEPP fractions, has not been assessed.

The present study aims to investigate a sustainable methodology for extracting polyphenols from red and white murta fruits and leaves and comprehensively analyzes their phenolic fractions, including EPPs and NEPPs. The following workflow was designed for this investigation: 1) various ES were formed, assessing their performance in conventional extractions based on EPP and flavonoid contents, antioxidant capacity, and yields of specific phenolics identified and quantified by HPLC-DAD; 2) the best performance ES was tested using MAE and UAE techniques at different time conditions; 3) the optimized procedure was used as a model for the analysis of remaining NEPPs in the extraction residues, to get a full characterization of these Chilean endemic materials.

2. Materials and methods

2.1. Chemicals

Pure (98–99.5 %) choline chloride (ChCl), betaine, 1,2-butanediol, 1,3-butanediol, and 1,2-propanediol were purchased from Sigma Aldrich (Burlington, VT, USA), and glycerol (99 %) was from Labquem (Madrid, Spain). Authentic standards (94–99 % purity) of gallic acid, 4-hydroxybenzoic acid, syringic acid, *p*-coumaric acid, caffeic acid, ferulic acid, quercetin, rutin, resveratrol, myricetin, cinnamic acid, and catechin were obtained from Sigma Aldrich (Burlington, VT, USA). The Folin-Ciocalteu (FC) reagent, sodium carbonate, aluminum chloride, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) high purity (98–99.5 %) were purchased from Merck (Darmstadt, Germany). HPLC grade (98–99.5 %) water, ethanol, acetonitrile, and acetic acid were obtained from Merck (Darmstadt, Germany). Chemical specifications such as CAS number, purity, supplier, abbreviation, or formula are detailed in supplementary material in Table S1. The chemical structures of the components of ES and polyphenol standards are also shown in supplementary material in Fig. S1.

2.2. Murta berries and leaves

Berries and leaves of wild white and red murta (*Ugni candollei* Barm, *Ugni molinae* Turcz) were collected in the forest of the Nahuelbuta Cordillera in April 2019 from Mahuilque (38°13′13.3″ S 73°15′19.5″ W), Biobío region of southern Chile. The collected material was dry-cleaned, and the fruits were separated and freeze-dried at 193 K (FDT-8620, Operon Co., Ltd., Gimpo, Republic of Korea). Finally, the dried samples were ground to 0.5 mm and stored at 253 K until extraction.

2.3. Preparation of ES

Eight ES were considered to perform the extractions containing ChCl or betaine acting as HBA and 1,2- butanediol, 1,3-butanediol, 1,2-propanediol, and glycerol acting as HBD. These ES were selected for the initial screening because previous works have shown positive results in terms of phenolics extraction (Ozturk et al., 2018; Wan Mahmood et al., 2019). ES were prepared in glass vials with a [HBA:HBD] molar ratio of 1:4. Both components were mixed at 353 K and 300 rpm for approximately 100 min until a clear and homogeneous solution was obtained. Then, 30 % (*v/v*) of water was added to each ES. Table 1 summarizes the compounds, molar ratios, and nomenclature.

2.4. Preparation of polyphenol fractions

The next workflow was designed to obtain the polyphenol fractions from murta: 1) evaluation of ES with orbital solid-liquid extraction; 2)

Table 1
Nomenclature, components, and molar ratios of ES.

Nomenclature	Compound HBA	Compound HBD	Molar ratio [HBA:HBD]
ChCl:1,2B	Choline Chloride	1,2-butanediol	[1:4]
Bet:1,2B	Betaine	1,2-butanediol	[1:4]
ChCl:1,3B	Choline Chloride	1,3-butanediol	[1:4]
Bet:1,3B	Betaine	1,3-butanediol	[1:4]
ChCl:1,2P	Choline Chloride	1,2-propanediol	[1:4]
Bet:1,2P	Betaine	1,2-propanediol	[1:4]
ChCl:Gly	Choline Chloride	Glycerol	[1:4]
Bet:Gly	Betaine	Glycerol	[1:4]

optimization of water content and feed:solvent ratio; 3) comparison of conventional extraction with MAE and UAE extractions; 4) selection of the optimized procedure as a model for the analysis of remaining NEPPs in the extraction residues. The workflow is resumed in Fig. 1.

2.4.1. Extractable polyphenols (EPP) fraction

2.4.1.1. Orbital extraction (OE). The extractions from fruits and leaves of white and red murta were carried out following a conventional solid-liquid extraction method (orbital extraction, OE). The dried murta samples were placed in contact with each of the eight ES (30 % *v/v* water:ES) and with a hydroalcoholic solution (30 % *v/v* water:EtOH) in an orbital shaking incubator (VorTemp 1550, Labnet, New Jersey, USA) during 100 min under the following conditions: 900 rpm, 333 K and a feed:solvent ratio of 1:10 (0.5 g of murta fruit or leaf and 5 g of ES). The extraction temperature was set at 333 K to avoid degradation of the phenolic compounds due to redox reactions, polymerizations, or hydrolysis (Cañadas et al., 2023).

2.4.1.2. Microwave-assisted extraction (MAE). The ES with the highest phenolic yield (the content of the polyphenols quantified by HPLC), also called the best-performing ES in OE (BP-ES), was selected for MAE extractions. Here, the samples (fruit and leaf of white and red murta) and the feed:solvent ratio of 1:10 *w/w* were mixed in a glass vial and placed in a microwave reactor (Monowave 400, Anton Paar, Graz, Austria) with a maximum power of 850 W. Extractions were carried out at 353 K and three different extraction times (1.5, 3, and 6 min), following previous literature (Jesus et al., 2023).

2.4.1.3. Ultrasound-assisted extraction (UAE). For UAE, an ultrasonic homogenizer (UP200Ht, sonotrode) made of titanium, with a diameter of 14 mm and approximate length of 80 mm (Hielscher Ultrasound Technology, Berlin, Germany) was employed. The feed:solvent ratio was 1:10 *w/w*, the probe's power was set at 10 W, and the extraction times were 1.5, 3, and 6 min (Jesus et al., 2023). Temperature control was not possible in this device; however, the temperature was recorded at the beginning and end of each extraction (323–373 K). All the extracts (OE, MAE, and UAE) were centrifuged at 4200 rpm for 15 to 30 min (Unicen 21, Ortoalresa, Madrid, Spain) to separate the solid and liquid phases. The liquid extracts were then analyzed using UV-VIS spectrophotometry and high-performance liquid chromatography (HPLC - DAD).

2.4.2. Non-extractable polyphenols (NEPPs) fraction

Residues of the best-performance EPP extractions were subjected to different treatments to obtain hydrolyzable polyphenols (HP) and non-extractable proanthocyanidins (NEPA) solutions. The total NEPPs content corresponded to the sum of the HP and NEPA contents obtained by spectrophotometry.

2.4.2.1. Hydrolyzable polyphenols. The residues from the EPP extractions were subjected to hydrolysis with methanol and sulfuric acid for 20 h at 358 K, and then the pH was adjusted to 5.5 (Hartzfeld et al., 2002; Pérez-Jiménez & Saura-Calixto, 2015). These hydrolysates were then subjected to a solid phase extraction (SPE) procedure with Oasis HLB cartridges (5400 mg, 3 cm^3, 30 μm) from Waters (Milford, MA, USA) to remove any salts that may damage the Mass Spectrometer (MS). After activation with pure methanol (5 mL) and 50 % methanol (5 mL), 1 mL of the sample was loaded and successively eluted with pure methanol (1 mL) and 80 % methanol (1 mL). The eluates were combined and concentrated under nitrogen.

2.4.2.2. Non-extractable proanthocyanidins. The EPP extraction waste was treated with butanol/HCl/$FeCl_3$ at 373 K for 1 h to release anthocyanins and xantylium compounds derived from butanolysis depolymerization (Reed et al., 1982; Zurita et al., 2012).

2.5. Polyphenol analysis

A selection of techniques was applied to EPP, HP, and NEPA, depending on the pros and cons of the different methodologies for each polyphenol fraction. The fractions where each technique was applied are detailed below.

2.5.1. Spectrophotometric analysis

2.5.1.1. Phenolic content in the different fractions. The phenolic content (PC) was determined in the three polyphenol fractions, *i.e.,* EPP, HP, and NEPA. In the case of EPP and HP, the Folin-Ciocalteau (FC) test was applied using the previously proposed FC reagent method (Singleton & Rossi, 1965) with some modifications for EPP and HP (Cañadas et al., 2021; Pérez-Jiménez & Saura-Calixto, 2008; Pérez-Jiménez & Saura-Calixto, 2015). Specifically, the sample was mixed with the FC reagent in a glass tube with a 1:1 *v/v* ratio. The mixture was homogenized and

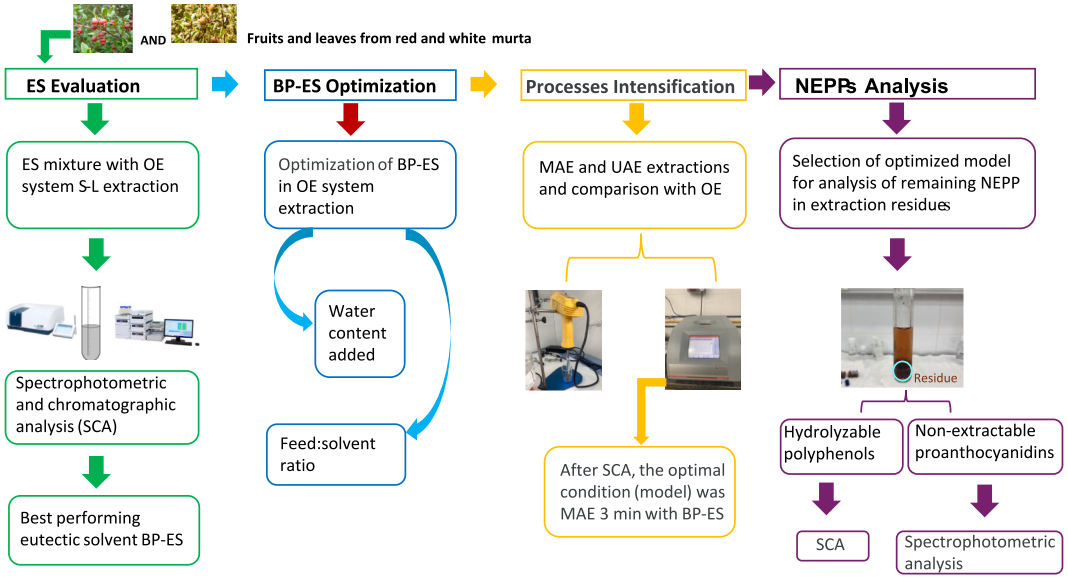

Fig. 1. Scheme of the different research steps carried out in this work. ES: eutectic solvents. OE: orbital extraction. SCA: spectrophotometric and chromatographic analysis. BP-ES: best performing eutectic solvent. MAE: Microwave-Assisted Extraction. UAE: Ultrasound-Assisted Extraction. NEPPs: non-extractable polyphenols.

allowed to stand for 3 to 8 min in the dark. A known volume of aqueous Na_2CO_3 solution was then added. The reaction was kept in the dark for a maximum of 60 min at room temperature. Subsequently, the absorbance at 765 nm was measured against a blank (blank of sample and solvents). Results were expressed as mg of gallic acid equivalent per gram of dried murta sample (mg GAE/g murta), using gallic acid as a standard (calibration curve of 10 to 100 mg/L).

Regarding NEPA, the absorbance of this fraction was measured at 555 and 450 nm (Pérez-Jiménez & Saura-Calixto, 2015). The results were compared with a proanthocyanidin standard from carob pod (*Ceratonia siliqua*), rich in high molecular weight proanthocyanidins (Reed et al., 1982; Zurita et al., 2012).

2.5.1.2. Extractable flavonoid content. Extractable Flavonoid content (EFC) in the EPP fraction was assessed following the methodology proposed previously by other authors (Cañadas et al., 2023; Zhishen et al., 1999). The reagents were added to a glass vial in the following order: 2 mL of distilled water, 150 μL of 5 % (*w/v*) Na_2NO_3, and 450 μL of sample. The mixture was shaken vigorously and incubated for five min at room temperature, followed by adding 150 μL of $AlCl_3$; the vials were shaken and allowed to stand for another five min. Then, 1 mL of 1 M NaOH was added to each vial to terminate the reaction. The reaction solution was mixed manually and kept at room temperature for 15 min. Subsequently, the absorbance of the reaction mixture was measured at 510 nm using the JASCO V-730 UV-VIS spectrophotometer (Tsukuba, Japan). Finally, the EFC in each extract was calculated using the calibration curve previously obtained with standard quercetin solutions at known concentrations (0–120 mg/L). EFC results were expressed in milligrams of quercetin equivalents (QE) per gram of dried murta (mg QE/g murta).

2.5.1.3. Antioxidant capacity. The antioxidant capacity (AC) of the EPP fraction was measured using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical assay (Brand-Williams et al., 1995; Wan Mahmood et al., 2019). A volume of 100 μL of sample was mixed with 2.9 mL of DPPH solution (6×10^{-5} mol/L in methanol), followed by a 30 min incubation in the dark at room temperature (298 K). Then, the absorbance of the resulting solution was measured at 515 nm using the JASCO V760 UV–Vis spectrophotometer (Tsukuba, Japan). The percent of absorbance inhibition of the DPPH·radical was calculated by comparing the test results with those of the control (methanol) using the following equation,

$$\%inhibition = \frac{(A_{blank} - A_{sample})}{A_0} x100$$

Trolox was used as a standard antioxidant, and the percentage of inhibition was measured for different concentrations of Trolox to construct a calibration curve. The AC was expressed in mg of Trolox equivalents per g of murta sample (mg TE/g murta).

2.5.2. Chromatographic analysis

2.5.2.1. HPLC – DAD analysis. Chromatographic analysis of the EPP fraction was performed on a JASCO 4000 Series HPLC system (Tsukuba, Japan) with a Fortis C18 column (250 mm × 4.6 mm, 5 μm, Neston, United Kingdom) and a photodiode array detector (DAD); measurements were carried out at wavelengths 214 nm, 280 nm, 320 nm, 365 nm, and 370 nm at room temperature. The method proposed by Sáenz de Miera et al. was followed (Sáenz de Miera et al., 2022). The compounds were separated with gradient elution using different ratios of 2 % (*v/v*) aqueous acetic acid solution (A) and acetonitrile (B). The conditions of the gradient method were as follows: 30 % of phase B initially; linear increase from 30 % to 35 % of B from 7 to 13.5 min; linear increase in B up to 55 % from 13.5 to 18.5 min; linear increase in B up to 60 % from 18.5 to 22.5 min; linear increase in B to 80 % from 22.5 to 25.5 min; and finally, linear decrease to 30 % of B between 25.5 and 30 min at a flow rate of 0.5 mL/min. Column re-equilibration was performed using initial conditions for 5 min before the next analysis. The total analysis took 35 min per sample. For identification and quantification, phenolic authentic standards were used: gallic acid, 4-hydroxybenzoic acid, syringic acid, *p*-coumaric acid, caffeic acid, ferulic acid, cinnamic acid, quercetin, rutin, myricetin, (+)-catechin, and resveratrol.

2.5.2.2. HPLC-ESI – QTOF analysis. Qualitative HPLC-MS analysis was performed on the HP fraction of the NEPPs (not on the NEPAs because hydrolysis degrades the original flavanol polymeric structures). An HPLC Agilent 1200 (Agilent Technologies, Santa Clara, CA, USA) with a DAD (Agilent G1315B), a QTOF mass spectrometer (Agilent G6530A), and atmospheric pressure electrospray ionization (ESI) were used for separation. The column used was Luna C18 100 A, 50 mm, 9.2 mm i.d., 5 μm (Phenomenex, Torrance, CA, USA) at 298 K. Gradient elution was performed with a binary system consisting of 0.1 % aqueous formic acid (solvent A) and 0.1 % formic acid in acetonitrile (solvent B). The following gradient was applied at a flow rate of 0.4 mL/min: 0 min, 8 % B; 10 min, 23 % B; 15 min, 50 % B; 20 min, 50 % B; 23 min, 100 % B; followed by a rebalancing step (García-Díez et al., 2022). The injection volume was 10 μL. Data were acquired using negative and positive ion modes with a mass range of 100 to 200 Da, using a source temperature of 598 K and a gas flow of 10 L/h. Peak identity was established by comparison with retention times of commercial standards (malvidin chloride, delphinidin chloride, cyanidin chloride, pelargonidin chloride, gallic acid, ellagic acid, 3-hydroxybenzoic acid, 4-hydroxybenzoic acid, 2,3-dihydroxybenzoic acid, syringic acid, ferulic acid, *p*-coumaric acid, caffeic acid, chlorogenic acid, (+)-catechin, (−)-epicatechin, procyanidin dimer B1, quercetin, quercetin-3-*β*-glucoside, isorhamnetin, myricetin, kaempferol) when available. Likewise, the molecular formula proposed by the Mass Hunter Workstation software, version 4.0 (Santa Clara, CA, USA), was compared for the different signals obtained in the MS experiments with phenolic compounds previously reported in murta fruits and leaves, considering a maximum error of 10 ppm.

2.6. Statistical analysis

In HP and NEPA fractions, a one-way statistical analysis of variance (ANOVA) with a Tukey *post hoc* test was conducted to identify significant differences ($p < 0.05$) between the different samples of murta. Previously, normal distribution and homogeneity of variance were evaluated. This analysis was performed using the IBM SPSS software version 29.

3. Results and discussion

3.1. Impact of ES mixtures on extractable polyphenols, flavonoids, and antioxidant capacity in the murta extracts

Murta extracts obtained with a conventional OE system, with eight different ES mixtures and a hydroalcoholic solvent as control, were subjected to the analysis of EPP content (Fig. 2A), extractable flavonoid content (Fig. 2B), individual polyphenols assessed by HPLC (Fig. 2C) and antioxidant capacity by DPPH assay (Fig. 2D). As a general trend, it was observed that polyphenol content (as total EPP, individual EPP, or EFC associated) was higher in the leaves than in the fruits, with similar values for red and white varieties. Concerning AC, no clear trend was observed since, for some ES, higher AC values were observed in fruits than in leaves. However, the highest values were obtained with the control solvent (70 % ethanol) in fruit and leaves. When comparing leaves and fruits, the same tendency was observed when antioxidant capacity was assessed by several methods (including DPPH assay) in *Rubus grandifolius* (Gouveia-Figueira & Castilho, 2015). In addition, it was observed that most of the murta EPP fraction corresponded to flavonoids, in accordance with previous literature (Avello-Lorca et al.,

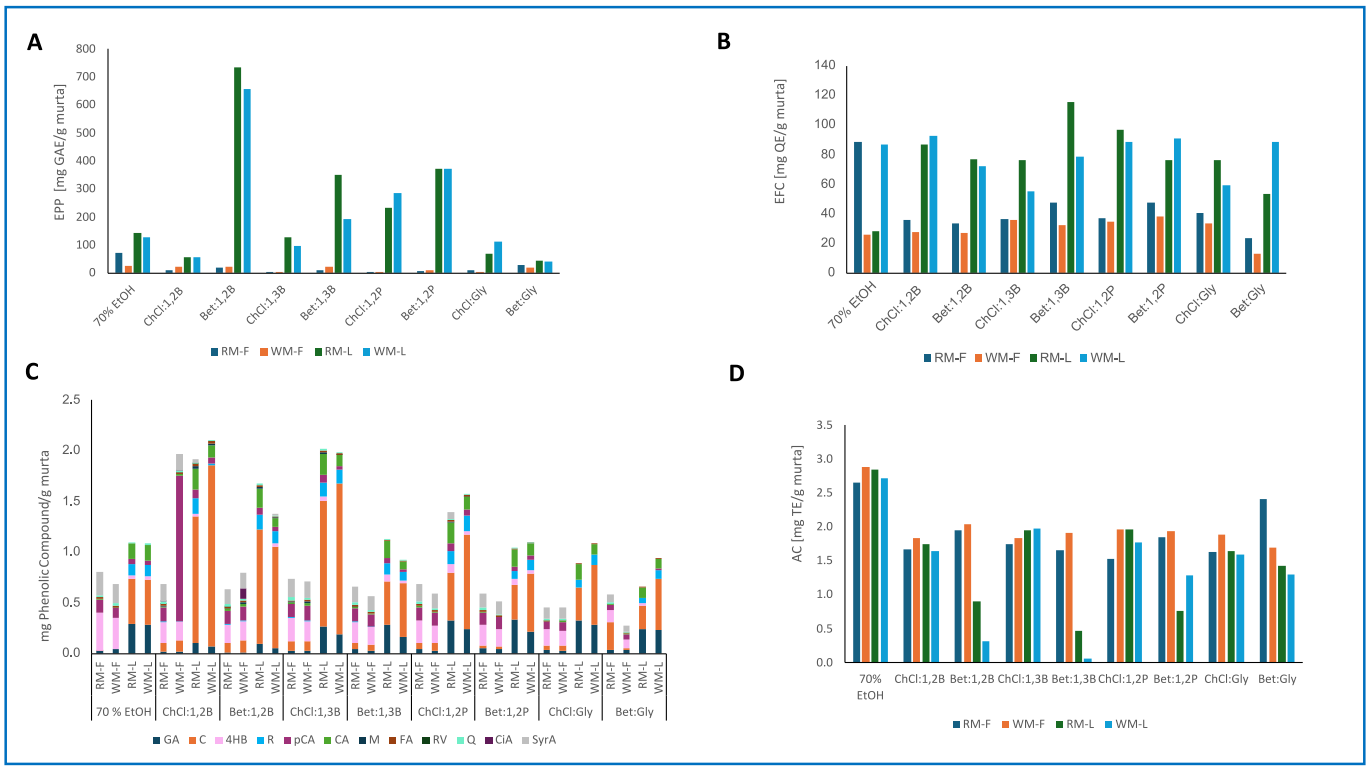

Fig. 2. A: Phenolic content (PC) in the extractable polyphenols fraction (EPP). B: Extractable flavonoids content (EFC). C: Cumulative phenolics compounds extracted from white and red murta. D: Antioxidant capacity (AC) of the EPP. All the fractions of white and red murta fruits and leaves were obtained using aqueous solutions of eight eutectic solvents or 70 % ethanol (70:30 ethanol:water, *v/v*). Extraction conditions: Orbital extraction (OE) system, 100 min, 333 K, feed:solvent ratio 1:10. RM: red murta, WM: white murta; F: fruit; L: leaves. GAE: gallic acid equivalent. QE: quercetin equivalent. GA: gallic acid; C: (+)-catechin; 4HB: 4-hydroxybenzoic acid; R: rutin; pCA: *p*-coumaric acid; CA: caffeic acid; M: myricetin; FA: ferulic acid; RV: resveratrol; Q: quercetin; CiA: cinnamic acid; SyrA: syringic acid. TE: Trolox equivalent. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2016; Fuentes-Jorquera et al., 2024). Nevertheless, the detailed EPP analysis by HPLC (Fig. 2C) showed that phenolic acids also contributed significantly to the total polyphenol content in these samples. The most abundant compounds in the fruits were 4-hydroxybenzoic acid, *p*-cumaric acid, and syringic acid, while gallic acid and (+)-catechin were the major ones in the leaf extracts.

No clear trend emerged from the solvent comparison, likely due to the diverse chemical structures within the phenolic compound family and the various factors that influence chemical extractions, such as diffusion coefficient, steric hindrance, and polarity. However, some ES mixtures can be selected as the most promising ones. For EPP content (Fig. 2A), ES did not exceed hydroalcoholic mixtures in murta fruit extracts compared with the control solvent (70 % ethanol). On the contrary, some ES were more effective in murta leaf extracts. In particular, betaine-based ES combined with 1,2-butanediol, 1,3-butanediol, or 1,3-propanediol, but not with glycerol, achieved higher EPP content values in leaf extracts. Similarly, Cañadas et al., in their investigation of antioxidant recovery from grape residues through ES extraction, indicated that using ES with Bet as the HBA component enhanced phenolic outcomes compared with ChCl (Cañadas et al., 2023). A clear trend is observed for the EFC (Fig. 2B), where the leaves have higher EFC contents than the fruits. Notably, the highest efficiency was observed in red murta leaves treated with the Bet:1,3B, which resulted in a 5.65-fold increase in extractable flavonoids compared to the solvent control for the same sample. A similar improvement in EPP content yields was achieved in the same matrix using the Bet:1,2B.

According to the HPLC analysis of the individual phenolic compounds in the EPP fraction (Fig. 2C), the recoveries of the different phenolic compounds were higher with ES based on ChCl than ES based on Bet. This difference was more notorious when ChCl was combined with 1,2-butanediol or 1,3-butanediol. It should be highlighted that some solvents were particularly efficient for extracting certain individual phenolic compounds, such as the hydroalcoholic solution for syringic acid or the mixture ChCl:1,2B for *p*-coumaric acid. Overall, ChCl-based ESs recovered more quantified polyphenols than Bet-based ESs. Such is the case of (+)-catechin, a flavonol with a structure that is based on the typical C6 – C3 – C6 flavonoid skeleton. Hydroxyl groups or gallate groups at the C3 position and ortho-dihydroxyl or vic-trihydroxyl groups on the B ring lead to the differences in structure and properties of catechin and its derivatives (Lang et al., 2024).

Furthermore, the formation of solvates, particularly hydrates, typically occurs in phenolic compounds such as (+)-catechin (Ashafaq et al., 2012). All of the above added to the fact that the higher the association between HBA and HBD, the lower the association between the ES solvents and the phenolic compounds (Gajardo-Parra et al., 2021). Therefore, the solubility of the polyphenols is lower. Bet-based ESs show greater interaction per molecule between HBA and HBD than ChCl-based ESs. The latter can self-associate because the Ch structure has a hydroxyl group (hydrogen bond donor) and is also a salt, so its constituents (Cl and Ch ions) are separated in the solution. This self-association reduces the possibility of interactions between HBA and HBD.

On the other hand, Bet molecules cannot self-associate due to their hydrophobic carboxylate molecule (Aravena et al., 2023). The partial discrepancies between values derived from colorimetric (Fig. 2A-B) and HPLC techniques (Fig. 2C) may be due to the interference of other non-phenolic substances. For example, the Folin assay is susceptible to multiple interferences, and several difficulties have been reported when applied to ES extracts (Percevault et al., 2021; Prior et al., 2005). Nevertheless, this assay was also included due to its generalized application for polyphenol screening.

Finally, according to the DPPH assay (Fig. 2D), ES extracts showed

lower antioxidant capacities than the control solvent extracts. Among ES, ChCl:1,3B and ChCl:1,2B achieved the maximum AC in fruits and leaf extracts, while Bet:Gly was particularly efficient for red murta fruits. It is important to be cautious when interpreting these results, as colorimetric methods are highly susceptible to interference, which can lead to inaccurate measurements. A precipitate formed during the preparation of ChCl-based eutectic solvents, likely resulting from interactions between the ionic nature of choline chloride and the colorimetric reagents.

Thus, based on an overall assessment of the results depicted in Fig. 2 and considering the pros and cons of each methodology, ChCl:1,3B was chosen as the best-performing ES (BP-ES) for carrying out the next steps of the previously described workflow.

3.2. Impact of water content and ratio of feed:solvent ratio in the EPP content of murta extracts

The next step to optimize the extraction of individual EPPs, measured by HPLC, was to test different amounts of added water (10 %, 30 %, and 50 %) on BP-ES dissolution (ChCl:1,3B) and different feed: solvent ratios (1:5, 1:10, and 1:20). The results of these experiments are shown in Fig. 3. The highest polyphenol content in all extracts was achieved with a water content of 30 %, *v/v* (Fig. 3A). The reduced capacity observed when more water is added may be due to the higher polarity of the extraction medium that reduces the polyphenolic solubilities (Huamán-Castilla et al., 2023). Additionally, excess water can break the hydrogen bonds between the ES components, causing the loss of its supramolecular structure (Grudniewska & Popłoński, 2020). Overall, the specific distribution of the different individual phenolic compounds remained similar in all extracts when 10 % and 50 % water content was added to the BP-ES compared to the previous extractions with 30 % water and the BP-ES (Fig. 2C).

Similarly, three feed:solvent ratios (1:5, 1:10, and 1:20) were tested with the OE method applied to murta leaf and fruit samples. The operating conditions were fixed, *i.e.,* 30 % of water in BP-ES (*v/v*), 333 K, and 100 min. Increasing the amount of solvent enhances the interactions between the biomass and the solvent. No phenolic compounds were detected when a 1:5 ratio was used (Fig. 3B); hence, this ratio was insufficient to achieve adequate contact between both phases, and the penetration of the ES into the cellular matrix was minimal or null (Wan Mahmood et al., 2019). On the other hand, although a maximum proportion of gallic acid was reached at a 1:20 ratio, specifically on leaf extracts, a lower overall extraction was observed when compared with the 1:10 scenario. Thus, the 1:10 ratio seems to be the optimal choice. Furthermore, as described by other authors, the 1:20 ratio generally results in a higher extractable phenolic content, but less concentrated polyphenols are obtained; thus, the use of higher amounts of solvent may not be economically viable (Ozturk et al., 2018). At the optimal condition, the overall distribution of individual phenolic compounds was similar to that observed in the experiments discussed above (Fig. 2C). In summary, the best conditions to test the advanced extraction techniques were solvent ChCl:1,3B, 30 % *v/v* water content, and 1:10 feed:solvent ratio.

3.3. Intensification of the extraction process by advanced techniques: microwave-assisted extraction (MAE) and ultrasound-assisted extraction (UAE)

The extraction conditions for murta fruits and leaves optimized in the previous experiments were tested in two advanced extraction systems, MAE and UAE, at three extraction times: 1.5, 3, and 6 min. The results of the HPLC analysis, polyphenols and flavonoids in the EPP fraction, and antioxidant capacity by DPPH assay are shown in Fig. 4.

Given the potential interferences that colorimetric methods may exhibit when dealing with ES solvents, as previously mentioned, the extraction yield was evaluated based on HPLC results (Fig. 4A). Overall, with the use of advanced extraction techniques, the distribution of the main phenolic compounds in the fruit and leaf extracts was similar to that observed in the above-mentioned experiments. Hence, notable

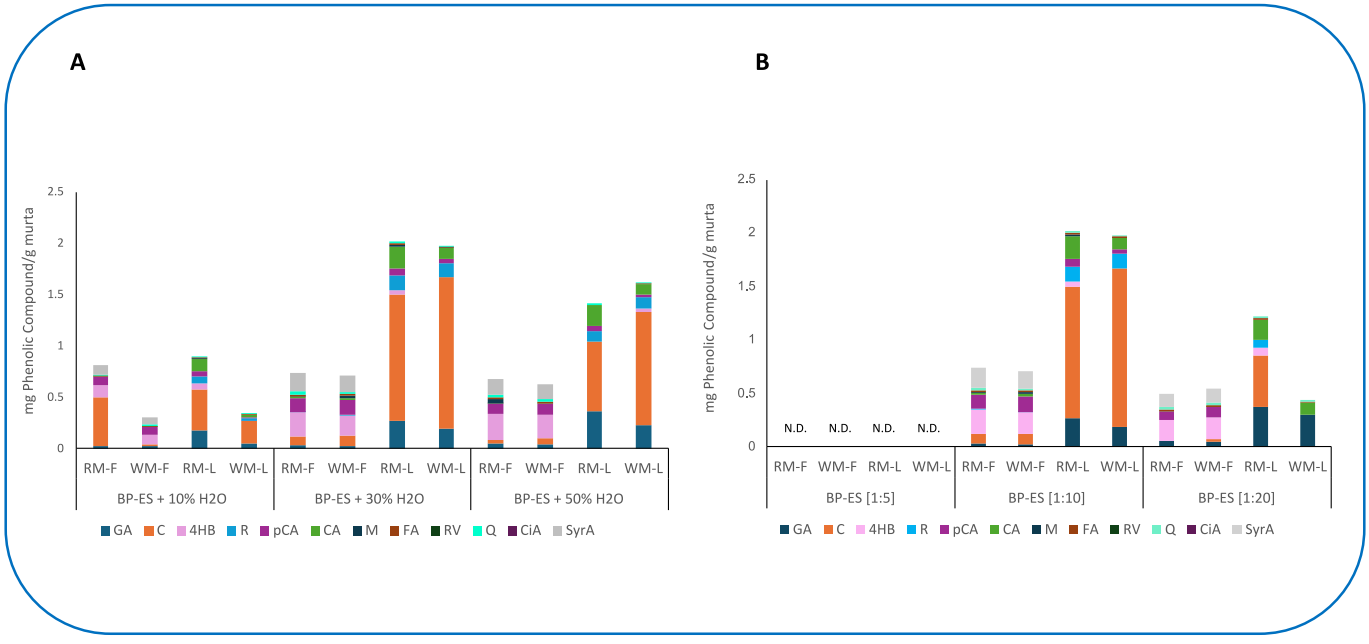

Fig. 3. A: Cumulative phenolics compounds in the extractable polyphenols (EPP) fraction with different water contents (10 %, 30 %, and 50 %, *v/v*) added to ChCl:1,3B, the best performing eutectic solvent (BP-ES). Extraction conditions: Orbital extraction (OE) system, 100 min, 333 K, feed:solvent ratio 1:10. B: Cumulative phenolic compounds in the EPP fractions using different feed:solvent ratios of 1:5, 1:10, and 1:20 with the BP-ES plus 30 % water content added (*v/v*). Extraction conditions: OE system, 100 min, 333 K. RM: red murta; WM: white murta; F: fruit; L: leaves; ChCl: Choline Chloride; 1,3B: 1,3-butanediol; GAE: gallic acid equivalent. QE: quercetin equivalent. GA: gallic acid; C: (+)-catechin; 4HB: 4-hydroxybenzoic acid; R: rutin; pCA: *p*-coumaric acid; CA: caffeic acid; M: myricetin; FA: ferulic acid; RV: resveratrol; Q: quercetin; CiA: cinnamic acid; SyrA: syringic acid. N.D.: not detected. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

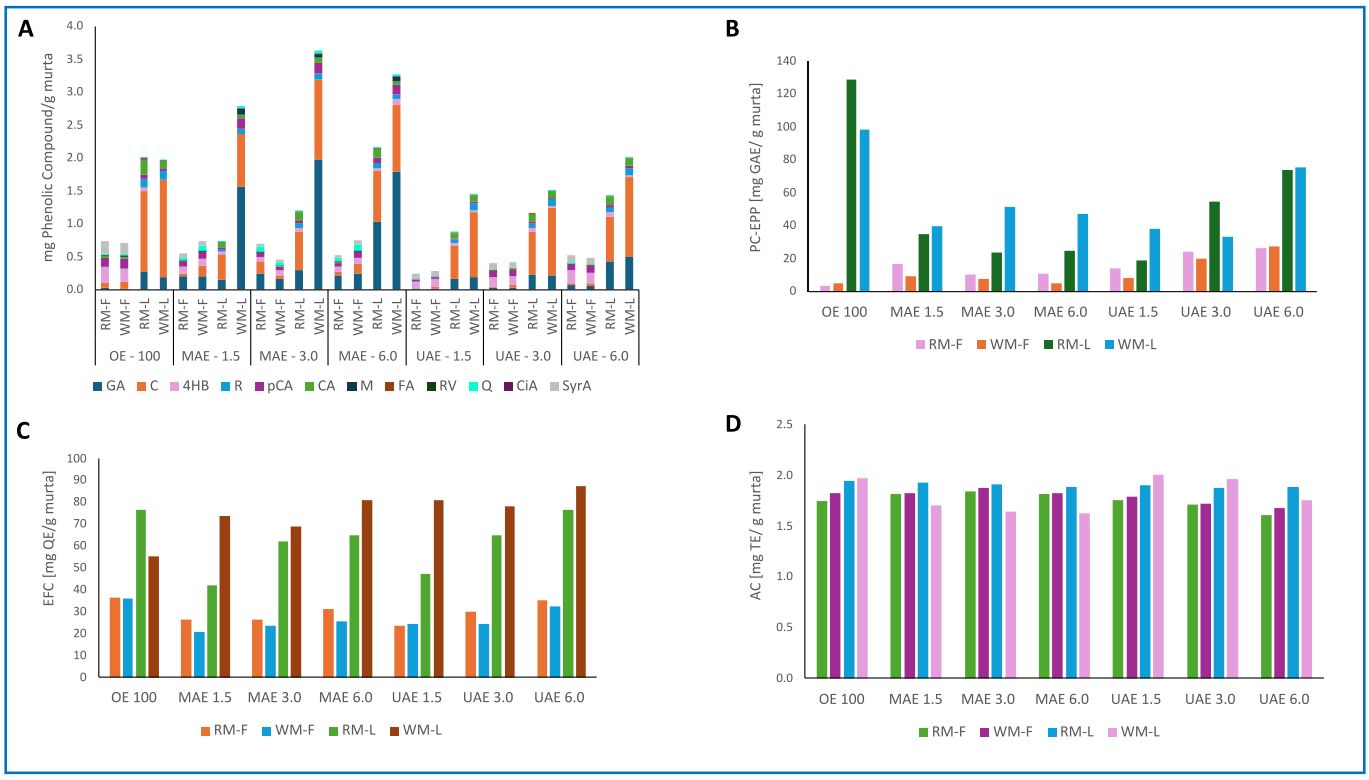

Fig. 4. A: Cumulative phenolics compounds in the extractable polyphenols (EPP) fraction using different advanced extraction techniques at 1.5, 3, and 6 min with the BP-ES. B: phenolic content in the EPP fractions. C: flavonoid content (FC) in the EPP fractions. D: Antioxidant capacity (AC) in the EPP fractions. Extraction conditions: OE system, 100 min, 333 K; MAE, 1.5, 3, and 6 min, 353 K. UAE, 1.5, 3, and 6 min, 323–373 K. RM: red murta; WM: white murta; F: fruit; L: leaves. ChCl: Choline Chloride; 1,3B: 1,3-butanediol. OE: orbital extraction. MAE: Microwave-Assisted Extraction. UAE: Ultrasound-Assisted Extraction. GA: gallic acid; C: (+)-catechin; 4HB: 4-hydroxybenzoic acid; R: rutin; pCA: *p*-coumaric acid; CA: caffeic acid; M: myricetin; FA: ferulic acid; RV: resveratrol; Q: quercetin; CiA: cinnamic acid; SyrA: syringic acid. TE: Trolox equivalent. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

differences can be observed within both techniques, showing that the MAE was more effective than the UAE. The optimum time for MAE was 3 min and 6 min for UAE. Both advanced extraction techniques showed higher or similar efficiency than the control method (OE, hydroalcoholic solution, 100 min) for murta leaves. In particular, MAE white murta leaf extracts showed higher content of the twelve extractable polyphenols than OE extracts. On the other hand, the control method (OE) seems to perform better than UAE for murta fruits.

It is known that the specific nature of the HBD used in the composition of an ES determines its properties. The ability to absorb microwaves depends on the system's HBD (Kyriakoudi et al., 2024). Previously, other researchers have tested the impact of various types of HBD in ES on microwave heating absorption (Wang, Jing, et al., 2020). The results indicated that, for alcohol-based ESs, the solvent temperature increased with prolonged irradiation time, while the overall microwave heating efficiency was reduced. The reduction in energy absorption by solvent molecules is likely attributable to the decline in viscosity. However, the heating rates are higher if the molar ratio of HBA:HBD favors HBD (*e.g.,* 1:4) since the polarity of the molecules increases. Consequently, the microwave absorption capacity of the ES is enhanced. For instance, González-Rivera et al. observed that MAE with an organic acid-based ES yielded a 2.6-fold higher quantity (in weight percent) of polyphenols extracted from chestnut shell waste than pure water when used as a solvent under identical extraction conditions (González-Rivera et al., 2021). The enhanced capabilities of the microwave process were validated in the study by Grillo et al., which compared the efficiency of MAE integrated with ES in the extraction of anthocyanins from blueberry peel. The optimized MAE-ES approach yielded a significantly higher output than conventional extractions conducted in acidified ethanol and reduced the total process time (Grillo et al., 2020). Similarly to MAE, UAE is an innovative extraction technique that reduces energy and solvent consumption and minimizes extraction duration while increasing extraction yield compared to conventional approaches (Kyriakoudi et al., 2024). The theoretical foundation of UAE with ES (UAE-ES) is based on the principles of ultrasonic cavitation and the properties of ES. The efficiency of UAE-ES extraction depends on several factors, including the nature of the ES used, the ultrasonic frequency, temperature, extraction time, and the plant material. These factors can influence the efficacy of UAE-ES (Siddiqui et al., 2023). For instance, Chanioti et al. employed ChCl as HBA and found that organic acid-based ES (citric acid and lactic acid) were more efficient in extracting phenolic compounds and other molecules from virgin olive pomace compared to sugar-based ES (maltose), polyol-based ES (glycerol), water, and aqueous ethanolic solvents. Water was added (20 %, *v/v*) to all ES, and the UAE technique operating conditions were employed at 313 and 333 K for 30 min (Chanioti & Tzia, 2018).

The differences were not marked in the total EPP content and flavonoids (Fig. 4B-C) when comparing MAE and UAE with the OE method. Even for EPPs extracted from murta leaves, OE seems to perform better. Overall, the advanced techniques were more efficient for recovering polyphenols from leaves of white murta than from murta fruits, which follows the trend observed for individual and total polyphenols (Fig. 4A). No differences were observed for the antioxidant capacity (Fig. 4D).

3.4. Non-extractable polyphenols in the residues of the optimized extraction

According to the results above, the optimal conditions for extracting polyphenols from fruit and leaves of murta were selected as follows: solvent ChCl:1,3B, MAE system, 3 min, and feed:solvent ratio of 1:10.

Table 2 shows the NEPP content in the residues of the optimized extraction conditions, comprising the previously described fractions, HP and NEPA. These compounds were found in significant amounts in the four samples, highlighting the need to consider them to analyze murta polyphenols comprehensively. Indeed, this agrees with other studies reporting a relevant amount of NEPPs remaining in the corresponding residues after extraction with advanced techniques (Domínguez-Rodríguez et al., 2017). The four samples had a very high NEPA content, between 8.2 ± 0.3 g/100 g dw for WM-F and 19.4 ± 2.6 g/100 g dw for WM-L. Notably, the NEPA value exhibited by WM-L (19.4 ± 2.6 g/100 g dw) was two-fold compared to the one obtained for a mixture of cocoa and carob, two of the vegetal materials with the highest NEPA content reported (García-Díez et al., 2022). The HP fraction represented more than 1 % for both fruits, similar to others with relevant HP content, such as apples (Pérez-Jiménez & Saura-Calixto, 2015). Notably, the HP content in the leaves was exceptionally high, reaching 3.9 ± 0.1 g/100 g in white murta and 5.4 ± 0.5 g/100 g in red murta.

It is possible to perform an individual analysis of phenolic compounds in HP once released from the food matrix since they keep their original structures (in contrast to NEPA, where the original structures have been depolymerized). The results of the HP after HPLC-ESI − QTOF MS analysis of the residues from white and red murta (both leaves and fruits) are shown in Table 3. Seven phenolic compounds were found in the leaf samples, while only four were identified in the fruit samples; this agrees with the higher HP contents found in leaves than in fruits (Table 2). The identified compounds correspond to 4 phenolic acids, a flavone, and two compounds belonging to other polyphenol classes, *i.e.,* 2,3-dihydroxy-1-guaiacylpropanone and ferulaldehyde. It has been suggested that ferulaldehyde can be generated as an artifact from other phenolic compounds during hydrolysis to release HP (Pérez-Jiménez & Saura-Calixto, 2015).

Although HP would not be absorbed in the small intestine, studies in other food matrices have shown that they may be transformed by human microbiota in the colon, contributing to the overall biological activities of polyphenols (Wang, Li, et al., 2020). Moreover, since this fraction of dietary polyphenols reaches the colon associated with dietary fiber, there is feedback between food constituents, and they contribute to the prebiotic effects of dietary fiber, as shown in *in vitro* and *in vivo* studies (Hou et al., 2024; Liu et al., 2020).

4. Conclusions

This study is inspired by applying sustainable methods, including ecological solvents, for extracting phenolic compounds from berries and

Table 2
Hydrolyzable polyphenols (HP) and non-extractable proanthocyanidins (NEPA) analyzed in the residues of optimal murta extracts using MAE with ChCl:1,3B 3 min, 353 K, ratio 1:10 (*w/v*). All fractions were obtained from fruits and leaves of red and white murta.

Sample	HP [g/100 g dw]	NEPA [g/100 g dw]
WM-F	0.9 ± 0.02[a]	8.2 ± 0.3 [a]
RM-F	0.8 ± 0.1[a]	12.0 ± 2.0 [a]
WM-L	3.9 ± 0.1 [b]	19.4 ± 2.6[b]
RM-L	5.4 ± 0.5 [c]	8.4 ± 0.3[a]

MAE: microwave-assisted extraction; ChCl:1,3B: choline chloride:1,3-butanediol. WM-F: white murta fruit; WM-L: white murta leaf; RM-F: red murta fruit; RM-L: red murta leaf. Different letters (a, b, and c) represent statistical differences ($p < 0.05$) between the types of samples for the concentration of HP or NEPA.

Table 3
Hydrolyzable polyphenols identified by HPLC-ESI − QTOF in the residues of optimal murta extracts: using MAE with ChCl:1,3B, 3 min, 353 K, ratio 1:10 (*w/v*). Hydrolyzable fractions were obtained from fruits and leaves of red and white murta.

Class	Subclass	Compound	Chemical Formula	*m/z* calc	WM-F		RM-F		WM-L		RM-L	
					m/z exp	Error	*m/z* exp	Error	*m/z* exp	Error	*m/z* exp	Error
Phenolic acids	Hydroxybenzoic acids	Gallic acid	$C_7H_6O_5$	169.0177	169.0184	−4.14	169.0178	−0.59	169.0189	−7.10	169.018	−3.55
	Hydroxybenzoic acids	Gallic acid ethyl ester/Syringic acid	$C_9H_{10}O_5$	197.0456	197.0451	2.28	197.0455	−2.54	197.0446	2.03	197.0457	−3.55
	Hydroxybenzoic acids/Hydroxyphenylacetic acids	Vanillic acid/3,4-Dihydroxyphenylacetic acid	$C_8H_8O_4$	167.0350	167.0332	10.66	167.0341	1.99	167.0357	−7.59	167.0360	−9.38
	Hydroxycinnamic acids	Ferulic acid	$C_{10}H_{10}O_4$	193.0506	n.d.		n.d.		193.0502	2.23	193.0525	−9.69
Flavonoids	Flavones	Tetramethylscutellarein	$C_{19}H_{18}O_6$	341.1025	n.d.		n.d.		341.1021	1.21	341.1040	−4.36
Other polyphenols	Hydroxybenzoketones	2,3-Dihydroxy-1-guaiacylpropanone	$C_{10}H_{12}O_5$	211.0606	n.d.		n.d.		211.0611	−2.14	211.0621	−6.88
	Hydroxycinnamaldehydes	Feruladehyde	$C_{10}H_{10}O_3$	177.0552	177.0545	3.78	n.d.		177.0538	7.73	177.0541	6.04

MAE: microwave-assisted extraction; ChCl:1,3B: choline chloride:1,3-butanediol. WM-F: white murta fruit; WM-L: white murta leaf; RM-F: red murta fruit; RM-L: red murta leaf, n.d.: not detected.

leaves of two varieties of native Chilean murta. The objective is to contribute to the existing knowledge of the polyphenolic profile of murta and to highlight the potential of its byproducts, such as the leaves, for further valorization since murta is an ideal functional food and a valuable addition to the bioeconomy of Chile.

ES, UAE, and MAE conditions were assessed for extracting polyphenols from white and red murta fruits and leaves. The selected conditions for the recovery of phenolic compounds using ES were ChCl:1,3B [1:4], 30 % *v/v* water content, and a feed:solvent ratio of 1:10, with advanced MAE extraction technique at 3 min. Under these conditions, a higher polyphenol content in murta leaves rather than in fruits was observed; differences between the main individual phenolic compounds were identified for both vegetal materials (*i.e.*, leaves and fruits), while similarities were detected between red and white murta varieties. Relevant NEPP content was found in the residues of the optimized extraction, comprising HP and, particularly, NEPA. These results show that the combination of ES with MAE is an efficient and sustainable approach for extracting phenolic compounds from murta fruits and leaves, although it should not be disregarded that a high NEPA content remains in the extraction residues.

CRediT authorship contribution statement

Natalia Fuentes-Jorquera: Writing – original draft, Investigation. **Marisol Villalva:** Writing – review & editing, Visualization. **Jara Pérez-Jiménez:** Writing – review & editing, Supervision, Conceptualization. **María González-Miquel:** Writing – review & editing, Supervision, Conceptualization. **Emilio J. González:** Writing – review & editing, Visualization. **María Salomé Mariotti-Celis:** Writing – review & editing. **José Ricardo Pérez-Correa:** Writing – review & editing, Supervision, Project administration. **Roberto I. Canales:** Writing – review & editing, Supervision.

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.ifset.2025.104017.

Data availability

Data will be made available on request.

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