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Comprehensive Characterization and Quantification of Antioxidant Compounds in Finger Lime (*Citrus australasica* L.) by HPLC-QTof-MS and UPLC-MS/MS

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Abstract: Australian finger limes (*Citrus australasica* L.), an unusual citrus due to its unique pulp with a caviar-like appearance, has reached the global market as a promising source of bioactive compounds that promote health. This research was, therefore, performed to shed light on the bioactivity and composition of different parts of *Citrus australasica* L. (peel and pulp). Initial ultrasound-assisted extraction using MeOH:H_2O (80:20, *v*/*v*) was carried out. After that, four fractions (hexane, ethyl acetate, butanol and water) were generated through liquid–liquid partitioning, and the total phenolic content (TPC) and antioxidant activity were evaluated using the Folin–Ciocalteu and the ferric reducing antioxidant power (FRAP) assays, respectively. The ethyl acetate fraction in the peel, which presented the highest values of TPC and antioxidant activity, was characterized using high-performance liquid chromatography coupled to quadrupole time-of-flight (HPLC-QTof) mass spectrometry. Fifteen compounds were identified, of which seven were characterized for the first time in this matrix. Moreover, ten phenolic compounds were quantified using ultra-performance liquid chromatography coupled with tandem mass spectrometry (UPLC-MS/MS). The major compounds in the sample were citric acid, pyrogallol, caffeic acid, coumarin, rutin, naringin, 2-coumaric acid, didymin, naringenin and isorhamnetin, which were found in a range from 2.7 to 8106.7 µg/g sample dry weight. Finally, the results presented in this novel work confirmed that the peel by-product of *C. australasica* L. is a potential source of bioactive compounds and could result in a positive outcome for the food, cosmetics and pharmaceutical industries.

Keywords: Australian finger limes; phenolic compounds; flavonoids; limonoids; antioxidant activity; HPLC; tandem mass spectrometry

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1. Introduction

Citrus is one of the most widely cultivated, processed and consumed fruits throughout the world [1], with an estimated production of 98 million metric tons in 2020–2021 [2]. It produces a large number of by-products (e.g., peels or seeds) that can cause, on one hand, severe environmental pollution [1] and, on the other hand, the loss of bioactive compounds that could be key components for developing nutraceuticals and functional foods. For that reason, and to allow the recovery of these potentially bioactive compounds from agri-food by-products, the circular economy is being widely promoted [3].

Citrus fruits belong to the genus of the flowering plants of the *Rutaceae* family, which is originally found in tropical and subtropical areas in Southeast Asia [4], whence it spread to the rest of the world [5]. Particularly, Australian finger limes (*C. australasica* L.), commonly

known as citrus caviar, are one of the seven native endemic bush species of Australia (Queensland) [6]. Due to isolation from the continent, this species evolved separately from the rest of the *Citrus* genus, showing specific morphological and unique features, such as long ovoid contour that resembles a shape of a finger, giving it its name. There are a wide variety of peel colours ranging from dark black to yellow or green, and the almost non-existent albedo or mesocarp when compared with other citrus species. The sacs are presented in independent spherical bubbles resembling caviar eggs in different colours, depending on the cultivars, as depicted in Figure 1.

Figure 1. *C. australasica* L. fruit used in the present study (detail of the spherical bubbles of the juicy sacs). Image owner: Ramon Aznar.

Today, *C. australasica* L. fruits have caught the attention of exclusive restaurants that can afford to use this special product to garnish seafood or add it to cocktails, and has even been reported to be an emerging new fruit flavour [7]. Despite very little information having been published around *C. australasica* L., its production was estimated to be around 10 tons per annum, and is expected to rise annually [8]. Interestingly, this citrus has also infiltrated the rest of the world, and it is grown even in the Mediterranean region [9]. The trend of growing and consuming *C. australasica* L. is forecasted to follow the same path as limes did a few decades ago, passing from being inexistent to being present in all the markets, and thereby becoming a staple fruit.

Besides their economic interest, citrus fruits are one of the top preferred food flavours of the world. They are valued due to their rich nutritive profile [5], and because they are an excellent source of bioactive compounds, such as phenolic compounds, which have shown health-salutary attributes, such as antimicrobial, antiviral, anti-inflammatory, antihypertensive or antioxidants effects [10–12]. Phenolic compounds that have been extensively reported in the citrus matrix are hesperidin, neohesperidin, narirutin, naringin, sinensetin, nobiletin and tangeretin [13–15]. Nevertheless, the chemical profile of *C. australasica* L. needs to be studied in depth, as it may have a different chemical profile to other citruses due to its adaptability to distinct agri-environmental conditions. For instance, Ruberto et al. have reported that *C. australasica* L.'s peel oil composition differed from another *Citrus* genus, except for limonoid and flavonoid derivatives [9]. A limited number of studies focused on evaluating the chemical composition of *C. australasica* L. have identified five terphenyl esters (limonene, citronellal, isomenthone, sabinene and α-phellandrene) [16,17], four phenolic compounds (cyanidin-3-glucoside and peonidin-3-glucoside [18], vanillic and caffeic acids) [19]. Due to the novelty of the matrix and its potential interest as a source of bioactive compounds, the aims of this work were: (1) to assess the antioxidant activity and the total phenolic content (TPC) of different fractions of *C. australasica* L. in peel and pulp, (2) to characterize for the first time the phenolic content of the most active fraction using high-performance liquid chromatography coupled to quadrupole time-of-flight (HPLC-QTof) mass spectrometry, and (3) to quantify the most relevant phenols using ultra-performance liquid chromatography coupled with tandem mass spectrometry (UPLC-MS/MS).

2. Material and Methods

2.1. Chemical and Reagents

Formic acid (MS grade), sodium carbonate (Na_2CO_3), 6-hydroxy-2,5,7,8-tetra methylchroman-2-carboxylic acid (Trolox), 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ), 0.22 µm polytetrafluorethylene (PTFE) filters, HPLC grade acetonitrile (ACN), methanol (MeOH), ethyl acetate (EtAc), butanol (BuOH) and *n*-hexane were obtained from Merck (formerly Sigma-Aldrich Co., Wicklow, Ireland). Authentic standards of the citric acid, pyrogallol, caffeic acid, gallic acid and rutin used for confirmation and quantification analysis were purchased from Merck (formerly Sigma-Aldrich, Co., Wicklow, Ireland) and coumarin, hyperoside, naringin, 2-coumaric acid, didymin, naringenin and isorhamnetin were procured from Extrasynthese (Genay, France).

2.2. Plant Material and Compounds Extraction

The fruit samples of *C. australasica* L. were supplied by MonCitric in October 2018, and were collected in an organic experimental field located in Cullera (Valencia, Spain, 39°13′0.2″ N 0°14′5.9″ W), a well-known citrus-growing area. Fruits (2 kg) were collected from different trees representative of the entire field.

The samples were frozen and transported to Teagasc (Ashtown, Ireland). The samples were then cleaned with Milli-Q water. Peel and caviar-like pulp from the fruits (Figure 1) were carefully separated, freeze dried and milled into a fine powder before their analysis. Exhaustive extraction was carried out in 80% aqueous methanol aided by ultrasonication, as outlined in Figure 2. Briefly, 1 g of the powdered material (peel or pulp) was extracted with 20 mL of methanol:water (80:20 (*v*/*v*)) in an ultrasound bath for 30 min at room temperature. This process was repeated three times to ensure the total extraction of phenolic compounds. Then, the three extractions were pooled, centrifuged and the methanolic solvent was evaporated in a rotavapor at 35 °C. Thereafter, the remaining water fraction (~12 mL) was successively liquid–liquid partitioned (×3) with 15 mL of *n*-hexane, ethyl acetate or butanol to obtain four different fractions for each matrix (peel and pulp).

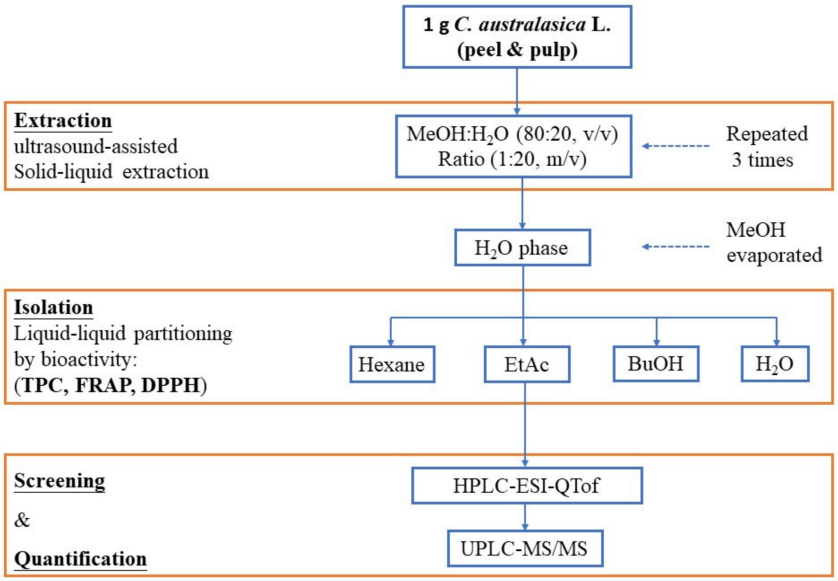

Figure 2. Flow chart of the extraction of phenolic compounds from *C. australasica* L.

2.3. Determination of Total Phenolic Content

The total phenolic content (TPC) of the resulting extracts was determined using the Folin–Ciocalteu assay [20,21] by measuring the absorbance at 735 nm using a plate reader (FLUOstar Omega Microplate Reader, BMG Labtech GmbH, Offenburg, Germany). In a 2 mL Eppendorf tube, 600 µL of water and 10 µL of sample were mixed with 50 µL of

undiluted Folin–Ciocalteu reagent. After 10 min, 150 µL of 2% Na_2CO_3 was added and topped up to 1 mL with distilled water. After 2 h of incubation at room temperature in the darkness, 200 µL of the mixture was transferred into a 96-well microplate. A standard curve was prepared as above, where the 10 µL sample was replaced with different concentrations of gallic acid (5–150 µg/mL). The TPC was calculated as mean ± SD (n = 3) and expressed as milligrams of gallic acid equivalent per hundred grams of dry weight of the extract (mg GAE/100 g d.w.).

2.4. Ferric Reducing Ability Power (FRAP) Assay

The antioxidant activity of the different extracts was assessed by the ferric reducing antioxidant power (FRAP) assay, previously described by Kenny et al., 2015 [22]. Briefly, a portion of an aqueous 10 mM solution of TPTZ reagent in 40 mmol/L of HCl was mixed with the same volume of 20 mmol/L $FeCl_3.6H_2O$ and a 10 times higher volume of acetate buffer (pH 3.6 prepared using 3.1 g sodium acetate and 16 mL acetic acid per litre). Then, the mixture was incubated at 37 °C for ten minutes, and 900 µL of the Fe^{3+}-TPTZ mixture and 20–50 µL of the sample were diluted up to 1000 µL with deionized water and incubated for at least 10 min. The same procedure was followed for the standards while water was employed for the blanks. After that, the absorbance was measured at 593 nm. One mmol/L of working solution of Trolox was prepared and used for calibration. The antioxidant capacity was calculated from the linear calibration curves, and results were expressed as milligrams of Trolox equivalent per one hundred grams of the dry weight of the extract (mg TE/100 g d.w.).

2.5. Characterization of the Most Active Fraction of C. australasica L. Using HPLC-QTof Mass Spectrometry

An Alliance 2695 HPLC system (Waters Corporation, Milford, MA, USA) coupled to a QTof Premier mass spectrometer was used to profile the potential bioactive compounds in the selected extract. Accurate mass measurements of the analytes and their fragment ions were achieved through the use of an internal reference compound (leucine enkephalin) that was introduced simultaneously. The analytes were separated on a T3 C_{18} Atlantis column (100 × 2.1 mm; 3 µm particle size) using Milli-Q water containing 0.1% formic acid (mobile phase A) and 0.1% formic acid in acetonitrile (mobile phase B) at a flow rate of 0.3 mL/min. The column temperature was maintained at 40 °C. The starting condition for each run was: 0 min, 10% B; 6 min, 40% B; 7 min, 50% B; 14 min, 70%; 16 min, 80% B held for 4 min. Electrospray mass spectra data were recorded using a negative ionization mode for a mass range m/z 70–1000. The capillary voltage and cone voltage were set at 3 kV and 30 V, respectively. Collision-induced dissociation (CID) of the analytes was achieved using argon at 12–20 eV energy.

2.6. Quantification of Phenolic Compounds Using UPLC-MS/MS

UPLC controlled by Acquity console software coupled to a mass spectrometer (Waters Corp., Milford, MA, USA) was used to quantify the different phenolic compounds present in the most active fraction. The separation of phenolic compounds was achieved using a Waters Acquity UPLC HSS T3 (100 × 2.1 mm, 1.8 µm), using Milli-Q water containing 0.1% formic acid (mobile phase A) and 0.1% formic acid in acetonitrile (mobile phase B) with a flow rate of 0.5 mL/min. The starting condition for each run was: 0 min, 2% B; 6 min, 10% B; 8.5 min, 50% B; 10 min, 70% and held for 3 min.

The mass spectrometer was operated in negative electrospray ionisation mode. The source temperature was set at 120 °C and the desolvation temperature at 350 °C. The capillary voltage was set at 2.8 kV. The cone voltages and collision energies were optimised for each of the compounds using IntelliStart™ software (Waters Corp.). Nitrogen gas was used as both sheath gas and auxiliary gas (800 L/h and 50 L/h, respectively). The detection and quantification of the phenolic compounds in the UPLC-MS/MS were conducted in multiple reaction monitoring (MRM) mode by analysing two or three transitions per compound

(Table S1, Supplementary Materials). Analyses were carried out in triplicate, and target compounds were quantified against the standard calibration curves of concentrations ranging from 10 ng/mL to 1 µg/mL. Phenolic compounds in *C. australasica* L. were expressed as µg/g of d.w. of citrus peel.

2.7. Statistical Analysis

All analysis (TPC, FRAP and UPLC-MS/MS) were performed in triplicate (n = 3). To evaluate differences at a 95% confidence level ($p \leq 0.05$) between antioxidant activity and the TPC of peel and pulp from the different extracted fractions, a one-way analysis of variance, Pearson's linear correlation and ANOVA (Tukey's honestly significant difference test with multiple comparisons) were employed using XLSTAT 2016.

3. Results and Discussion

3.1. TPC and Antioxidant Activity

In general, the peel of the *C. australasica* L. showed significantly ($p < 0.05$) higher TPC and antioxidant activity than the pulp, as depicted in Figure 3 and Table S2, and as recently described by Adhikari et al., who compared the TPC in peel and pulp of four Florida-grown finger limes (*Citrus australasica* F. Muell.) [23]. Considering the content of phenolic compounds in the different extracted fractions, the ethyl acetate fraction of peels showed the highest TPC, followed successively by the butanol and aqueous fractions (265.95 ± 0.04 vs. 215.16 ± 0.09 and 175.49 ± 0.06 mg GAE/100 g d.w., respectively) whereas the hexane fraction presented the lowest content in phenolic compounds (63.43 ± 0.02 mg GAE/100 g d.w.). Those differences could be influenced by the fact that the phenolic compounds are moderately polar and they tend to accumulate in the medium-polarity fraction, such as ethyl acetate, as previously described by other authors [24]. Consequently, the high phenolic content was reflected in the antioxidant activity, where the ethyl acetate fraction of *C. australasica* L. peels also showed the highest FRAP reducing activity, i.e., 176.43 ± 0.15 mg TE/100 g d.w., compared to the other extracted fractions, exhibiting also the hexane fraction with the lowest antioxidant activity (0.20 ± 0.00 mg TE/100 g d.w.). A higher antioxidant activity in the peel compared to the pulp has been previously reported in different varieties of finger limes by Wang et al. [25], and could be attributed to the fact that peels are directly exposed to biotic and abiotic stresses that trigger the biosynthesis of phenolic compounds [26,27]. Thus, the ethyl acetate fraction of *C. australasica* L. peel was chosen from the phenolic compounds for further investigation using mass spectrometry techniques.

3.2. Chemical Characterization of C. australasica Peel Using HPLC-QTof-MS

The elucidation of the compounds in each chromatographic peak was achieved through the analysis of the candidate formula with a mass accuracy limit of 5 ppm. The characterization strategy was based on several factors: on the accurate MS, their CID fragments and the use of authentic standards for those that were commercially available, as well as with information available in the existing literature. The SciFinder Scholar (http://scifinder.cas.org, accessed on 15 October 2021), MassBank (http://massbank.jp, accessed on 15 October 2021), METLIN Metabolite (http://metlin.scripps.edu, accessed on 15 October 2021) and the Food (https://foodb.ca/, accessed on 15 October 2021) databases were employed for the acquisition of chemical structural information. As many as 22 different compounds were detected in the ethyl acetate fraction, of which 15 were tentatively identified in the *C. australasica* L. peel (Figure 4 and Table 1). Table 1 shows the retention time (RT) of each peak, the experimental m/z of deprotonated molecular ions ($[M - H]^-$), the molecular formula, the error (ppm), the main MS/MS fragments and the tentative identification for each compound/peak shown in the chromatogram. These compounds were numbered according to their elution time. Additionally, the compounds identified for the first time in *C. australasica* L. peel are in bold. The identified compounds belonged to different chemical classes from the phenolic compounds' families, i.e., seven flavonoids

and derivatives, three phenolic compounds derivatives classified as other polyphenols, two phenolic acids and derivatives, two limonoids and one organic acid.

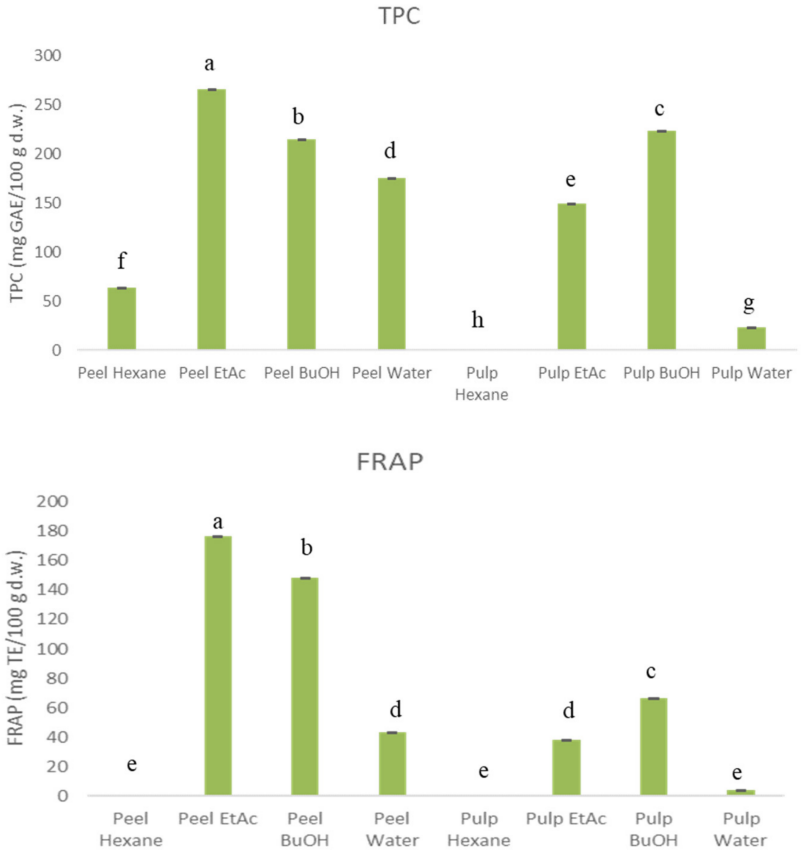

Figure 3. Total phenolic content (TPC) and antioxidant activity of *C. australasica* L. peel and pulp fractions were obtained using different solvents. Data are expressed as mean ± standard deviation and mean values are provided on top of each bar. Mean values with different superscript letters are significantly different ($p < 0.05$). Data shown in Table S2.

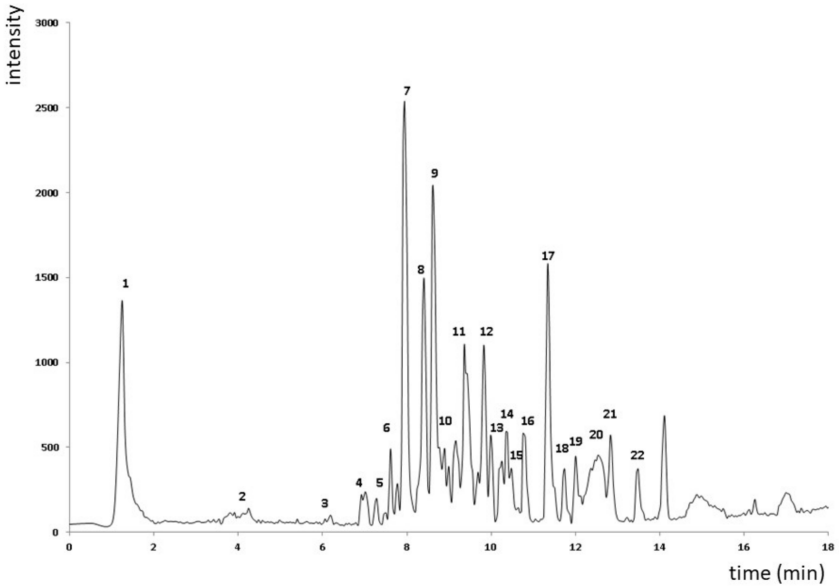

Figure 4. Base–peak chromatogram (BPC) of ethyl acetate fraction of *C. australasica* L. peel using HPLC-QTof-MS.

Table 1. Identification of phytochemicals in the ethyl acetate fraction of *C. australasica* L. using HPLC-QTof-MS.

Peak	RT (min)	$[M-H]^-$ *m/z* Experimental	$[M-H]^-$ *m/z* Calculated	Molecular Formula	Error (ppm)	MS/MS Fragment ions (*m/z*)	Proposed Compound
1	1.3	191.0176	191.0192	$C_6H_8O_7$	−1.6	160, 111, 87	Citric acid *
2	3.8	145.0274	145.0290	$C_9H_6O_2$	−1.6	117	Coumarin *
3	6.1	179.0341	179.0344	$C_9H_8O_4$	−0.3	135	Caffeic acid *
4	7.0	119.0496	119.0497	C_8H_8O	−0.1	101	**4-Vinylphenol**
5	7.3	609.1471	609.1456	$C_{27}H_{30}O_{16}$	1.5	301	Rutin *
6	7.6	463.0877	463.0877	$C_{21}H_{20}O_{12}$	0	301	**Hyperoside ***
7	8.0	579.1732	579.1714	$C_{27}H_{32}O_{14}$	3.1	271, 151	Naringin *
8	8.4	315.0498	315.0505	$C_{16}H_{12}O_7$	−2.2	301, 273, 151	Isorhamnetin *
9	8.6	163.0407	163.0395	$C_9H_8O_3$	1.2	119	**2-Coumaric acid ***
10	9.1	471.1268	471.1291	$C_{24}H_{24}O_{10}$	−4.9	267, 205, 163, 59	**Ononin-O-acetate**
11	9.4	593.187	593.1870	$C_{28}H_{34}O_{14}$	0	431, 285, 163,	Didymin *
12	9.8	503.1871	503.1858	$C_{33}H_{28}O_5$	3.6	355, 297	Unknown
13	9.9	491.2245	491.2222	$C_{33}H_{32}O_4$	4.7	373, 329, 285, 165	Unknown
14	10.2	271.0612	271.0606	$C_{15}H_{12}O_5$	2.2	171, 151	Naringenin *
15	10.4	125.0229	125.0239	$C_6H_60_3$	−1	81	**Pyrogallol ***
16	10.8	489.2107	489.2125	$C_{26}H_{34}O_9$	−3.7	471, 333, 111	**Deacetylnomilinic acid**
17	11.3	517.2073	517.2074	$C_{27}H_{34}O_{10}$	−0.2	399, 274, 175, 111	Unknown
18	11.7	469.1872	469.1862	$C_{26}H_{30}O_8$	2.1	381, 229	**Limonin**
19	12.0	333.1355	333.1338	$C_{18}H_{22}O_6$	4.1	191, 149	Unknown
20	12.5	445.2934	445.2954	$C_{27}H_{42}O_5$	−4.5	367, 287, 227	Unknown
21	12.8	425.2345	425.2328	$C_{26}H_{34}O_5$	4	410, 381, 335, 311, 283	Unknown
22	13.5	467.2452	467.2434	$C_{28}H_{36}O_6$	3.9	381, 325, 283, 179, 97	Unknown

* Compounds identified with commercial standards. Compounds identified for the first time in *C. australasica* L. peel are in bold.

Thirteen phenolic compounds and derivatives were tentatively identified in the *C. australasica* L. peel. Among them, the identification of two phenolic acids, i.e., caffeic acid (peak 3) and 2-coumaric acid (peak 9), were confirmed using commercial standards. Although coumarin has been widely detected in other citrus fruits [28], and other coumarins in *C. australasica* L. [29], this is the first time that this compound has been reported in *C. australasica* L. In addition, seven flavonoids were identified in the studied matrix. Flavonoids are one of the most common phenolic compounds found in citruses [30–32]. This has been reflected also in *C. australasica* L. samples where rutin (peak 5, *m/z* 609.1456), hyperoside (peak 6, *m/z* 463.0877), naringin (peak 7, *m/z* 579.1714), isorhamnetin (peak 8, *m/z* 315.0505), didymin (peak 11, *m/z* 593.1870) and naringenin (peak 14, *m/z* 271.0606) were identified using the commercial standards (Table 1). Finally, peak 10, with a [M-H]$^-$ ions at *m/z* 471.1291, was tentatively identified as ononin-O-acetate on the basis of its fragmentation pattern, which included main fragments at *m/z* 307, 266 (formononetin), 205, 163 and 59 (acetate functional group), and this is depicted in Figure S1 (Supplementary Materials). Ononin has been reported in four *Citrus* species (namely, *C. aurantinum*, *C. grandis*, *C. limonia* and *C. sinensis*) via HPLC-MS-SIM, while ononin-O-acetate has only been identified in *Citrus aurantium* so far [33]. However, it is the first time that this flavonoid derivative compound was identified in *C. australasica* L. peel.

Four compounds were characterized among the group of other polyphenols. In this regard, coumarin (peak 2, $[M-H]^-$ ions at *m/z* 145.0274) and pyrogallol (peak 15, $[M-H]^-$ ion at *m/z* 125.0229) were identified using commercial standards as highlighted in Table 1. Peak 4, with molecular formula C_8H_8O, was tentatively characterized as vinylphenol. Its main fragment yielded at *m/z* 101 corresponds to the loss of one molecule of water. Vinylphenols, in particular 4-vinylphenol and 4-vinylguaiacol, have been documented in citruses such as blood orange or in *Citrus aurantium* L. bloom [34], but not in the studied matrix.

Two limonoids, well-known compounds from the *Citrus* genus, were also detected in *C. australasica* L. peel (peaks 16, 17, 18 and 22). Peak 16, with molecular formula $C_{26}H_{34}O_9$, and which, according to its fragmentation pattern, included main fragments at *m/z* 471 $[M-H-H_2O]$ and 333 $[M-H-C_4H_4O-2CO_2]^-$ [35], was tentatively identified as deacetylnomilinic acid. This limonoid derivative has been earlier described in citruses [35,36], but it is the first time that this compound has been detected in *C. australasica* L. peel. Meanwhile, peak 18 was tentatively identified as limonin. Importantly, it presents

an $[M - H]^-$ ion at m/z 469.1862 with MS/MS fragments at m/z 381 and 229, which have been previously reported in the literature [35], and characterized in the seeds of grapefruit and the pulp of *Citrus* Sp. [37] and in *Citrus grandis* [35]. This limonoid, present in the *Rutaceae* family in which citrus fruits are included, has been found to have anticancer properties [18].

Lastly, peak 1 ($[M - H]^-$ ions at m/z 191.0192) was characterized as one of the most well-known compounds present in all citrus fruits, i.e., citric acid [38]. The identification of this organic acid was confirmed by comparison with the authentic standard.

3.3. Quantification of Polar Compounds from C. australasica L. Peel Using UPLC-MS/MS

The citrus industry generates a large amount of waste, and the use of its by-products as a source of nutraceuticals and natural food additives are of high priority [39]. Indeed, the by-products in citrus may have the ability to revolutionize the functional food industry [40]. Therefore, the accurate identification and quantification of the novel matrices of compounds of interest is a must. Fifteen standard calibration curves for quantifying the main potential bioactive compounds found in *C. australasica* L. peel extract were prepared using the following available commercial standards: citric acid, pyrogallol, caffeic acid, rutin, coumarin, hyperoside, rutin, naringin, 2-coumaric acid, didymin, naringenin and isorhamnetin. The calibration curves had good linearity between different concentrations ranges, with regression coefficients near 0.999 in all cases. The quantitative results are presented in Table 2.

Table 2. Quantification of phenolic compounds in *C. australasica* L., expressed as µg compound/g sample d.w. (n = 3).

Compound	Average ± SD
Citric acid	8106.7 ± 180.8
Pyrogallol	22.5 ± 2.2
Caffeic acid	11.2 ± 7.4
Coumarin	5.4 ± 2.8
Hyperoside	n.q.
Rutin	8.7 ± 1
Naringin	916.3 ± 75.4
2-Coumaric acid	97.4 ± 10.1
Didymin	111.8 ± 19.1
Naringenin	2.7 ± 0.2
Isorhamnetin	41.9 ± 3.4

n.q. = not quantifiable. Data are expressed as average ± standard deviation (SD).

Citric acid was the most abundant compound quantified (8106.7 µg/g sample d.w.), and was also previously detected and quantified in the same matrix, but at higher concentrations, in fresh weight (f.w.), ranging between 46,800 and 58,800 µg/g f.w. [41], which could be explained due to the study of different cultivars [25], where it was reported to be between 8260 and 20,750 µg/g f.w. Citric acid is abundant in C. *australasica* L., marking this fruit as a potential source of organic acids [23]. After citric acid, the second most abundant compound was the flavonoid naringin (916.3 µg/g sample d.w.), which is commonly present in all citrus fruits, such as pomelo (3910 µg/g f.w.) or grapefruit, at higher concentrations, though this is dependent on the method of extraction (1800 to 2800 µg/g d.w. for conventional extraction and 2400 to 3600 µg/g d.w. for ultrasound-assisted extraction) [32,42]. They are of interest because they have been found to exhibit strong antioxidant and anti-inflammatory activities, and several lines of investigation suggest that the use of naringin as a food supplement may be of benefit to treat obesity, diabetes, hypertension and metabolic syndrome [43]. Didymin was the second most abundant flavonoid, which was found at 111.8 µg/g sample d.w. It has been previously detected in citrus peel (rinds) at values around 400 µg/g d.w [44]. Currently, it is being studied to treat lung cancer in vitro and in vivo [45] and gastric cancer [46]. Isorhamnetin and rutin (41.9 and 8.7 µg/g sam-

ple d.w., respectively) are compounds extended in citruses and which present anticancer properties [47], among other health benefits. Pyrogallol was present at 22.5 μg/g sample d.w. and it has also been quantified in other citruses, such as C. *aurantium*, but in bloom and at a higher concentration (541.27 μg/g d.w) [34]. Only trace levels of caffeic acid were detected in the studied matrix, which is in agreement with previous work conducted on several citrus matrices (but finger limes were not studied) [48]. Coumarin compounds are generally distributed throughout the citrus species [49]. In this species, coumarin and 2-coumaric acid were quantified at 5.4 and 97.4 μg/g d.w, respectively.

3.4. Structure Bioactivity

A good correlation between the phenolic content and antioxidant activity (FRAP) data was observed (R^2 ranging from 0.884 to 0.994 in peel and pulp, respectively). Phenolic compounds that possess antioxidant activity are known to be mainly phenolic acids and flavonoids [50], and citrus matrices are very rich in these sub-classes of polyphenols [10,12]. Phenolic acids are the major classes of phenolic compounds and in this matrix, several have been identified and quantified in *C. australasica* L. peel, such as caffeic and coumaric acid. Among the phenolic compounds, flavonoids are the main polyphenols present in a wide variety of plant sources, including citrus fruits [50], which is also reflected in this study (Table 2). In general, the antioxidant activity of these compounds has been attributed to their electron-donating ability, mainly due to the hydroxyl groups, which are essential for their effective free-radical scavenging capacity [51].

4. Conclusions

This paper represents a first approach in evaluating the TPC and antioxidant activity of different fractions of *C. australasica* L. peel and pulp. One of the goals of this work has been the characterization of the potential bioactive compounds in *C. australasica* L. using HPLC-QTof-MS, allowing the identification of fifteen compounds, seven of them described for the first time in the studied matrix. It has proven that *C. australasica* L. peel is a good source of citric acid, 2-coumaric acid and flavonoids, such as naringin and didymin, among other phenolic compounds. The novel data presented in this work will encourage the further study of the *C. australasica* L. peel and other by-products for their revalorization as a source of bioactive phytochemicals which could be used as ingredients for functional foods, cosmetic, pharmaceutical and nutraceutical formulations.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/app12031712/s1, Table S1: UPLC-MS/MS MRM conditions for phenolic compounds; Table S2: Total phenolic content (TPC) and antioxidant activity of *C. australasica* L. peel and pulp fractions were obtained using different solvents; Figure S1: MS/MS fragments of ononin-O-acetate (peak 10) found in the EtAc fraction of *C. australasica* L.

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