REVALORIZATION OF *PASSIFLORA* SPECIES PEELS AS A SUSTAINABLE SOURCE OF ANTIOXIDANT PHENOLIC COMPOUNDS

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ABSTRACT

Food industry generates a big amount of residues. Nowadays, there is interest in adding value to these residues with the aim of increasing the sustainability of the food chain and to reduce the environmental impact of this waste whose revalorization could also originate an economical benefit. Passion fruits are cultivated for juice and pulp production generating high amounts of vegetable residues. The scarce information about passion fruit peels confers a high interest to the study of their phenolic profiles. In this work, an efficient extraction method based on pressurized hot water extraction was employed to obtain antioxidants from four *Passiflora* species peels (*P. ligularis*, *P. edulis*, *P. edulis* flavicarpa and *P. mollissima*). Antioxidant properties of the extracts were tested by *in vitro* assays and intracellular reactive oxygen species scavenging. *P. mollissima* and *P. edulis* peel extracts presented higher antioxidant capacity and phenolic content than *P. ligularis* and *P edulis* flavicarpa. Tentative structural elucidation of 57 phenolics was achieved by high-performance liquid chromatography-quadrupole-time of flight mass spectrometry. Flavones, chalcones and phenolic acids were the polyphenol classes that may contribute to antioxidant capacity of the *Passiflora* peel.

Keywords: antioxidants; HPLC-DAD-QTOF/MS; *Passiflora*; passion fruit by-products; phenolic compounds; pressurized hot water extraction.

1. INTRODUCTION

Residues from the food industry are causing an important environmental problem since their removal requires special treatments due to their high organic load. Besides, residue treatments represent an important economic expenditure by the food industry (Morais Ribeiro et al., 2014). Thus, in order to improve the economic benefits and decrease the negative environmental problem generated, the interest of revalorization of food by-products is promoted studying different healthy substances that can be extracted to be used in the elaboration of functional foods and nutraceutical supplements (Morais Ribeiro et al., 2014; Corrêa et al., 2016). In this sense, the juice industry provides a huge volume of passion fruits by-products (Corrêa et al., 2016). In fact, 65-70% of the total weight of the fruits remains as residues, mainly corresponding to seeds, peels and leaves (Ishimoto et al., 2007; Corrêa et al., 2016). Indeed, several studies have suggested that these by-products are an important source of bioactive compounds for the production of natural products with high added value, although they still have been scarcely studied (Morais Ribeiro et al., 2014).

Passion fruits are popular fruits from the genus *Passiflora* L. which has numerous plants distributed in tropical and subtropical regions in the world (Ángel-Coca et al., 2011). Within these species, the passion fruit variety *Passiflora edulis* Sims f. flavicarpa Degener (maracujá or yellow passion fruit) and *Passiflora edulis* Sims f. edulis (gulupa or purple passion fruit) are highly appreciated because of their edible fruits (Ángel-Coca et al., 2011). However, *Passiflora ligularis* Juss (granadilla) and *Passiflora mollissima* (Kunth) Spreng (banana passion fruit) are less known possibly due to climate constraints that limit the production of these varieties (Simirgiotis et al., 2013; Saravanan et al., 2014). The edible part and by-products of *Passiflora* fruits have shown high antioxidant capacity (Figueiredo et al., 2016). In general, the antioxidant capacity of passion fruits and their by-products has been attributed to their content in phenolic compounds (Sasikala et al. 2011).

Phenolic compounds from plants are commonly extracted by solid-liquid extraction (SLE) at different temperatures and with different extraction solvents (Zibadi et al., 2007; Betim Cazarin et al., 2016). Nevertheless, this conventional extraction technique requires large amounts of solvents and long extraction times. Thus, advanced extraction techniques have emerged to extract phenolic compounds and to enhance the aspects above mentioned, providing short extraction times with small amounts of solvents and an automatic extraction (Zekovic et al., 2016). The extraction of phenolic compounds from *Passiflora* species has mostly been performed by SLE using different extraction solvents such as water, methanol, ethanol and mixtures of these solvents sometimes acidified with trifluoroacetic acid and HCl (Kidoy et al., 1997; Zeraik et al., 2010; Simirgiotis et al., 2013; Betim Cazarin et al., 2016). As far as our knowledge goes, there is just one work that employed the advanced extraction technique called pressurized liquid extraction (PLE) with 64% ethanol (v/v) as extraction solvent at 80 °C with five cycles of 10 min each cycle in order to extract phenolic compounds from leaves of different *Passiflora* species (Gomes et al., 2017). In the present work, PLE was used for the extraction of phenolic compounds from *Passiflora* peel. Water was used as extraction solvent and this extraction technique is called pressurized hot water extraction (PHWE). In PHWE, the extraction process is more efficient applying high temperatures due to faster diffusion rates that combined with high pressures enhances diffusion within the sample matrix improving the extraction yield in relation to conventional extraction techniques such as SLE. Besides, water is a respectful extraction solvent with the environment with interesting chemical and physical properties to extract bioactive compounds (Plaza et al., 2015).

Phenolic compounds have scarcely been studied in *Passiflora* peels. Flavanols (catechin or epicatechin), flavonols (kaempferol 3-*O*-glucoside), flavones (luteolin-8-C-neohesperidoside) or anthocyanidins (cyanidin-3-*O*-glucoside) (Zibadi et al., 2007) have been identified in *P. edulis* through HPLC-DAD, as well as, isoorientin and isovitexin in *P. edulis* flavicarpa peel

extracts (López-Vargas et al., 2013). Additionally, flavones have been identified by HPLC-DAD-ESI-MS/MS in *P. mollissima* and *P. edulis* peel extracts such as isoorientin, orientin, isovitexin, vitexin, schaftoside and vicenin-2 (Zucolotto et al., 2012; Simirgiotis et al, 2013). Nevertheless, to our knowledge phenolic compounds from *P. ligularis* have not been described. Considering the lack of information on the characterization of phenolic compounds from the different species of passion fruits peels, there is a need for a more detailed examination of these fruit peels to provide a more integrated assessment of their polyphenolic potential and its exploitation. Therefore, the main aim of this work was to revalorize the food residue *Passiflora* peel using a green extraction technique as PHWE in order to obtain extracts rich in antioxidant phenolic compounds. To achieve this aim, the extract collected by PHWE from four different *Passiflora* species was characterized concisely by reversed phase (RP)-high-performance liquid chromatography (HPLC) with photodiode array detector (DAD) and electrospray ionization (ESI) quadrupole-time-of-flight (QTOF) mass spectrometry (RP-HPLC-DAD-ESI-QTOF-MS). Additionally, the total phenol content and antioxidant capacity (DPPH and ABTS assays), and the intracellular reactive oxygen species (ROS) scavenging capacity were measured and the contribution of the different classes of phenolic compound to the total antioxidant capacity was studied.

2. MATERIALS AND METHODS

2.1. Chemical and reagents

Ethanol, acetonitrile (99.9%) and formic acid (98-100%) of HPLC grade were purchased from Scharlab Chemie (Barcelona, Spain) and methanol (99.99%) from Fisher Scientific (Leicestershire, UK). Gallic acid, sodium carbonate, Folin-Ciocalteau reagent, 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (trolox), potassium persulfate, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH•), 3-[4,5-dimethylthiazol-2-yl]-2,5- diphenyltetrazolium bromide

(MTT), dimethyl sulfoxide (DMSO), trypsin, propidium iodide, antibiotics (penicillin, streptomycin, and amphotericin), fetal bovine serum, and Dulbecco's Modified Eagle's Medium (DMEM) were obtained from Sigma-Aldrich (Saint Louis, MO, USA). Tertbutylhydroperoxide (TBHP), and 2', 7'-dichloro-dihydrofluoresceindiacetate (H_2DCFDA) were acquired at Invitrogen (Barcelona, Spain).

Dipotassium hydrogen phosphate and sodium dihydrogen phosphate dihydrate were purchased from Merck (Darmstadt, Germany).

Acetonitrile and formic acid of LC-MS grade were obtained from Fisher Scientific (Leicestershire, UK). Ultrapure water (18.2 MΩ/cm) was generated with a Millipore system (Millipore, Billerica, MA, USA).

2.2. Plant material

Four different species belonging to the Passifloraceae family and the *Passiflora* L. genus were chosen for this study: *Passiflora edulis* Sims edulis (passion fruit), *Passiflora ligularis Juss* (granadilla) and *Passiflora tripartite* var. *mollissima* (banana passion fruit) from Colombia, and *Passiflora edulis* Sims flavicarpa (yellow passion fruit) from Ecuador. The fruits samples banana passion fruit and granadilla were obtained from a local market/shop in Medellín, Colombia, and passion fruit and yellow passion fruit were bought at a local market in Alcalá de Henares, Madrid, Spain. Different fruit pieces from each *Passiflora* species were washed, manually peeled, mixed, freeze-dried, grounded in a commercial blender and stored at -20 ºC until their analysis.

2.3 Pressurized hot water extraction of phenolic compounds

Extractions were carried out in a Dionex ASE 150 instrument (Thermo Fisher; Germering, Germany). Extraction of freeze-dried Passiflora peel samples was achieved in 10 mL extraction cells, which were filled with 2 g of cleaned sand and 1 g of solid sample for P. *edulis* flavicarpa, *ligularis* and *mollissima*, while for P. *edulis* the cells were filled with 2 g of cleaned sand and

0.5 g of solid sample. The extraction solvent mixture of water/ethanol/formic acid (94:5:1, vol%) was sonicated for 30 min for removing dissolved oxygen. Extractions were performed at 99 ºC and 1500 psi for 1 min based on an optimized method employed for the extraction of anthocyanins from red cabbage (Arapitsas & Turner 2008). Prior to each experiment, the cell was heated-up for 6 min. Samples were prepared in triplicate. The Passiflora peel extracts were freeze-dried and stored at -20 ºC until their analysis.

2.4 Total phenols and antioxidant capacity determination

2.4.1 Total phenolic content (TPC)

In order to determine the total phenolic content, the Folin-Ciocalteau (FC) method based on the protocol by Kosar et al. (2005) with some modifications was applied (Plaza et al. 2017). The results were expressed as mg of gallic acid equivalents (GAE)/g extract.

2.4.2 DPPH radical scavenging assay

DPPH method was applied according to Brand-Williams, Cuvelier & Berset (1995) with some modifications (Plaza et al. 2013). The percentage of remaining DPPH was plotted on a graph against the extract concentration in order to obtain the concentration required to decrease the initial DPPH concentration by 50% (EC_{50}). Therefore, the lowest the value, the highest the antioxidant capacity.

2.4.3 Trolox equivalent antioxidant capacity (TEAC) assay

The TEAC assay described by Re et al. (1999) with some modifications was employed (Plaza et al. 2013). Trolox was employed as reference standard, expressing the results as TEAC (trolox equivalent antioxidant capacity) values (mmol trolox/g extract). The TEAC values were obtained from four different concentrations of each extract giving a linear response between 20 and 80% comparing with the initial absorbance.

2.5 Cell culture and treatments

164 Human cervical cancer HeLa cells, obtained from the American Type Culture Collection
165 ATCC (Rockwell, MD, USA) were cultured in Dulbecco's modified Eagle's medium (DMEM)
166 supplemented with penicillin (100 U/mL), streptomycin (100 µg/mL), amphotericin (250
167 ng/mL) and 10% of fetal bovine serum. The cells were maintained under 37 °C, 5% CO_2 and
168 95% of humidity in their culture medium.

169 **2.6 Cell viability**

170 The effect of different concentrations of *Passiflora* extracts on cell viability was measured
171 using the MTT assay described by Hernández-Corroto et al. (2018). Different concentrations
172 *Passiflora* extracts were diluted before in DMEM culture medium (25, 100, 400, 700 and 1000
173 µg/mL). Cell viability was calculated by the following equation:

174 $$\%\ cell\ viability = \frac{Abs\ sample - Abs\ control}{Abs\ control} \times 100$$

175 **2.7 Intracellular reactive oxygen species (ROS) scavenging assay**

176 The analysis was performed by measuring the fluorescence intensity of the H_2DCFDA assay,
177 which was proportional to the amount of ROS formed according to Hernández-Corroto et al.
178 (2018). Cervical cancer HeLa cells were treated with different concentrations of *Passiflora*
179 extracts (1000, 700, 400, 100 and 25 µg/mL) dissolved in DMEM medium. DMEM medium
180 without oxidizing reagent was employed as control and TBHP were added as positive control
181 to generate oxidative stress. Trolox antioxidant (1 mg/mL) was used to compare its capacity
182 with the *Passiflora* extracts from peels. Results were expressed as % ROS production which
183 was calculated as follow:

184 $$\%\ ROS\ production = \frac{FI\ sample - FI\ control}{FI\ TBHP - FI\ control} \times 100$$

185 where FI is fluorescence intensity due to ROS formation.

186 **2.8 Identification of phenolic compounds by high-performance liquid chromatography**
187 **with diode array and mass spectrometry detection (HPLC-DAD-MS)**
188 The analysis of all phenolic compounds in the different *Passiflora* peel extracts was performed
189 using an HPLC system 1100 from Agilent (Agilent Technologies, Palo Alto, CA, USA)
190 equipped with a diode array detector (DAD) and connected to a quadrupole-time of flight mass
191 spectrometer (QTOF/MS) Agilent 6530 equipped with an orthogonal electrospray ionization
192 (ESI) source (Agilent Jet Stream, AJS). The HPLC instrument was equipped with a quaternary
193 solvent pump, an auto-sampler, and a column heater compartment. Agilent Mass Hunter
194 Qualitative Analysis Software B.07.00 from Agilent was employed for HPLC and MS control,
195 data acquisition, and data analysis.
196 The chromatographic separation was carried out by using a porous-shell fused-core Ascentis
197 Express C18 analytical column (150 × 2.1 mm, 2.7 μm particle size) with an Ascentis Express
198 C18 guard column (0.5 cm × 2.1 mm, 2.7 μm particle size), both from Supelco (Bellefonte,
199 PA, USA). The mobile phases consisted of (A) water with 0.5% of formic acid (50 mM, pH
200 2.4), and (B) acetonitrile with 0.5% of formic acid (50 mM) in a gradient elution analysis
201 programmed as follows: 5% B (0-10 min); 5 to 40% B (10-50 min); 40 to 5% B (50-51 min),
202 with 15 min of post-time. The injection volume, flow rate, and column temperature were 5 μL,
203 0.3 mL/min and 50 ºC, respectively. The detection wavelengths used were 200, 280, 350, and
204 520 nm. The mass spectrometer operated in positive and negative ion mode in full scan mode
205 from mass range of m/z 100 to 1700. MS parameters were the following: capillary voltage,
206 3000 V; nebulizer pressure, 25 psig; drying gas flow rate, 10 L/min; gas temperature, 300 ºC.
207 The fragmentor voltage (cone voltage after capillary) was set at 175 V. The skimmer and
208 octapole voltages were 60 V and 750 V, respectively. Source sheath gas temperature and flow
209 were 300 ºC and 6.5 L/min, respectively. MS/MS was performed employing the auto mode and
210 the following conditions; 2 precursors per cycle, dynamic exclusion after two spectra (released

after 1 min), and collision energy of 5 V for every 100 Da. Internal mass calibration of the instrument was carried out using an AJS ESI source with an automated calibrant delivery system. Analyses were carried out in triplicate for each extraction.

2.9 Statistical analysis

The statistical program Statgraphics Centurion XVII (Statistical Graphics Corp., USA) was employed for statistical analysis. Analysis of variance (ANOVA) by Fisher´s exact test to discriminate on the least significant difference LSD ($p \leq 0.05$) which was used to compare differences in *Passiflora* species of antioxidant effect, total phenolic content, cytotoxicity and intracellular ROS scavenging capacity. Besides, a correlation between *in vitro* antioxidant and intracellular ROS scavenging capacities and individual phenolic compounds and their groups identified by HPLC was established by Pearson test ($p \leq 0.05$). Data were presented as mean ± standard deviation of nine measurements from three extracts. ~~All analyses were carried out in triplicate.~~

3. RESULTS AND DISCUSSION

3.1. Extraction of phenolic compounds from *Passiflora* peels from different species

In order to carry out the extraction of phenolic compounds from the four different *Passiflora* species peel, pressurized hot water extraction (PHWE) was employed. *P. edulis* has high amount of anthocyanins that for chemical stability reasons require acidic conditions and lower extraction temperature (Kidoy et al., 1997). Thus, in this work, formic acid was employed as assistive in the extraction solvent in order to lower the pH (pH 2.0). The extraction conditions used to extract phenolic compounds from *Passiflora* peel were based on a previous optimized study to extract phenolic compounds from red cabbage (Arapitsas et al., 2008) and on the PHWE conditions generally employed to achieve the extraction of phenolic compounds (Plaza et al., 2015). After PHWE of polyphenols from four different *Passiflora* species peel, *P. edulis*, *P. edulis* flavicarpa, *P. ligularis* and *P. mollissima,* the extracts were ready for further analysis.

236 The obtained extracts were subsequently studied in terms of *in vitro* antioxidant capacity, total
237 phenolic content, and intracellular ROS scavenging capacity and they were characterized
238 chemically in order to know their exact composition and to correlate both.

239 **3.2 Antioxidant capacity**

240 The antioxidant assays can be classified in two groups: hydrogen atom transfer- and electron
241 transfer-based assays. Hydrogen atom transfer-based assays evaluate the capacity of an
242 antioxidant to quench free radicals by hydrogen atom donation, whereas the electron transfer-
243 based assays consist of measuring the ability of an antioxidant to transfer one electron to reduce
244 a compound. In this work, the most used *in-vitro* antioxidant methods based in electron transfer,
245 DPPH and TEAC assays, were employed due to their speed, simplicity, applicability and low
246 cost in comparison with other antioxidant methods (Ishimoto et al., 2012). The use of two
247 different antioxidant capacity methods can contribute to a deeper knowledge of the chemical
248 composition of the extracts as well as their diverse capacities against different radicals.

249 The results obtained using these procedures are summarized in **Table 1**. It is important to
250 consider that the results from the DPPH method were expressed as EC_{50} (µg freeze dried
251 extract/mL) (effective concentration to inhibit 50% of the radical) and therefore, the lowest the
252 value, the highest the antioxidant capacity. As it is shown in **Table 1**, both assays gave related
253 results.

254 The results among *Passiflora* species were statistically different ($p \leq 0.05$) in both assays.
255 Considering DPPH assay, *P. mollissima* and *P. edulis* peel extracts showed the highest
256 antioxidant capacity with EC_{50} values of 10.56 ± 0.80 and 32.93 ± 2.88 µg extract/mL,
257 respectively (**Table 1**). Meanwhile, *P. edulis* flavicarpa generated the less active extracts with
258 an EC_{50} value of 718.91 ± 40.55 µg extract/mL. DPPH is the most reported method to analyze
259 the antioxidant capacity of *Passiflora* species. In general, the scarce information available in

the literature from *Passiflora* peel extracts showed lower antioxidant capacities compared to this study (Sasikala et al., 2011).

Regarding TEAC assay (**Table 1**), in accordance with the DPPH assay, *P. mollissima* extracts showed the highest antioxidant capacity (2.24 ± 0.15 mmol Trolox/g extract) with statistically significant differences among species ($p \leq 0.05$). Nevertheless, *P. ligularis* extracts showed the lowest antioxidant capacity of the study (0.05 ± 0.01 mmol Trolox/g extract) along with *P. edulis* flavicarpa (0.08 ± 0.01 mmol Trolox/g extract).

Few studies have been done on antioxidant capacity using the ABTS method in *Passiflora* species peel. The results obtained are within the TEAC value ranges found in literature (Sasikala et al., 2011).

The variations between DPPH and TEAC assays are due to the fact that in each method different compounds of the sample react with the radical employed (Domínguez-Rodríguez et al. 2017). Consequently, a combination of antioxidant capacity assays is recommendable to be used in order to obtain more accurate results.

3.3 Total phenolic content (TPC)

In order to discover potential correlations between the chemical composition of the different *Passiflora* peel extracts and their antioxidant capacity, the total amount of phenolic compounds was measured employing the FC assay.

Table 1 shows the TPC measured by FC assay for four different *Passiflora* extracts obtained by PHWE. As it can be seen, TPC values among *Passiflora* species were statistically different ($p \leq 0.05$). In addition, TPC values obtained ranged from 5.08 to 30.19 mg GAE/g extract. The richest extract in terms of total ~~phenols~~ phenolic compounds was *P. mollisima* with a TPC value of 30.19 ± 3.01 mg GAE/g freeze dried extract, followed by *P. edulis* and *P. edulis* flavicarpa. In contrast, *P. ligularis* showed the lowest content, with a TPC value of 5.08 ± 0.48 mg GAE/g freeze dried extract extract.

285 TPC values obtained for peel from different passion fruits in this study were higher than the
286 ones found in the literature (Infante et al. 2013).

287 **3.4 Effect of *Passiflora* species extracts on viability and level of intracellular oxidative**
288 **stress in HeLa cells**

289 **Figure 1A** shows the cytotoxic capacity of *Passiflora* extracts at four different concentrations
290 (25-1000 μg/mL) on HeLa cell cultures. At the concentrations used, cell viability was not
291 significantly altered ($p \geq 0.05$) which means that the *Passiflora* extracts under these
292 concentrations did not present cytotoxicity. As far as we know, there are not published studies
293 about the cytotoxic effect of *Passiflora* peel extracts on cell viability.

294 In addition, **Figure 1B** displays the effect of *Passiflora* extracts on the prevention of
295 intracellular ROS formation, which was measured by flow cytometry. Results showed that the
296 intracellular ROS production under the oxidative compound TBHP significantly decreased (p
297 ≤ 0.05) when *P. edulis* and *P. mollissima* extracts were added. However, *P. ligularis* and *P.*
298 *edulis* flavicarpa did not significantly decrease the ROS production ($p \geq 0.05$). As can be
299 observed in **Figure 1B**, the most active extract was that from *P. mollissima* because this extract
300 inhibited the formation of ROS at low concentrations (25 μg/mL extract). The reduction of
301 intracellular ROS production at the four different concentrations of *P. mollissima* extracts was
302 40.5 ± 8.2 % and no statistical differences were observed among the different concentrations
303 ($p \geq 0.05$). Also, *P. edulis* presented the ability of reducing the intracellular ROS production to
304 43.8 ± 2.0 % at the extract concentration of 400 μg/mL being this concentration higher than
305 that from *P. mollissima* extract. There were not significant differences ($p \geq 0.05$) in the capacity
306 of *P. edulis* to decrease the intracellular ROS production at higher concentrations of 400 μg/mL
307 extract. However, *P. ligularis* and *P. edulis* flavicarpa did not show a significant decrease ($p \geq$
308 0.05) on the intracellular ROS production at the tested extract concentrations. In order to
309 compare the capacity of intracellular ROS production of *Passiflora* extracts with a recognized

antioxidant compound, the synthetic antioxidant Trolox at a concentration of 1000 µg/mL was used. Trolox solution reduced the intracellular ROS production capacity to 50.0 ± 5.6 %, while *P. mollissima* presented a reducing capacity around 40.5-34.1 %, *P. ligularis* of 23.4-13.9 %, *P. edulis* of 43.8-9.5 %, and *P. edulis* flavicarpa of 17.8-0 %. In this sense, *P. mollissima* and *P. edulis* were the most active extracts to inhibit ROS production with inhibition values close to synthetic antioxidant (Trolox).

3.3 Phenolic profiling of *Passiflora* species extracts by HPLC-DAD-QTOF-MS and MS/MS

The phenolic profiling of the aqueous extracts from peels of *Passiflora* species was carried out by employing RP-HPLC-DAD coupled to a QTOF-MS and MS/MS equipped with an orthogonal electrospray ionization (ESI) source. An optimization of the separation process of phenolic compounds by HPLC was achieved using different composition of mobile phases (water with 0.5% of formic acid (solvent A) and acetonitrile with 0.5% of formic acid (solvent B); and water with 0.5% of formic acid (solvent A) and methanol with 0.5% of formic acid (solvent B)), gradient program (gradient time, gradient shape, and initial composition of the mobile phase), column length (100 mm and 150 mm), column temperature (40 ºC and 50 ºC) and detection wavelength (200, 280, 350, 370 and 520 nm). The final results showed that the best resolution and shortest analysis time were achieved with the separation conditions described in section 2.5. The careful analysis of the separated compounds, using the information provided by the DAD detector (wavelengths of 280 nm for the identification of the phenolic acids, flavanols and chalcones; 350 nm for the identification of flavonols and flavones; and 520 nm for the identification of anthocyanidins) for a preliminary classification of phenolic compounds, as well as the MS and MS/MS spectra which allowed to get their molecular formula and fragmentation patterns, together with the information that could be found in the literature and the MS databases (FOODB and PhytoHub) enabled the tentative

identification of 12, 15, 11 and 20 phenolic compounds from *P. ligularis*, *P. edulis*, *P. edulis* flavicarpa and *P. mollissima* peel extracts, respectively. **Table 2** shows the data for 57 phenolic compounds detected using mainly negative ionization mode, for the following *Passiflora* species, peak assignment number, proposed assignment name, retention time, experimental m/z (monoisotopic ion), molecular formula, error (ppm), main MS/MS fragments, UV/vis absorption maxima and the MS score that is based on mass error, isotope abundance and isotope spacing for the proposed molecular formulas (it was set at ≥ 83, except for the compound 14 of *P. edulis* (artemitin) which presented a MS score of 73.62). The negative ESI ionization conditions employed for the detection of the phenolic compounds did not allow the identification of the main peak (peak 6) and other minor peak (peak 14) present on the extracts of *P. edulis* (**Figure 2B** and **Table 2**). With the aim to identify these phenolic compounds, positive ESI ionization analysis was carried out.

By combining the information of their MS spectra and MS/MS fragmentation patterns, it was possible to significantly increase the certainty of the tentative assignments. As shown in **Figure 2** and in **Table 2**, almost all the main peaks separated in HPLC-DAD-ESI-QTOF/MS analysis of *Passiflora* species extracts could be tentatively identified.

The genus *Passiflora* is known to contain mainly flavonoids that include apigenin, luteolin, quercetin and kaempferol. However, vicenin, orientin, isoorientin, vitexin, isovitexin, lucenin-2, shaftoside and violanthin are the most characteristic in different *Passiflora* peels (Simirgiotis et al., 2013; Betim Cazarin et al., 2016) as well as in *Passiflora* leaves (Zucolotto et al., 2012) and pulp (Zeraik et al., 2010).

3.3.1. *Passiflora ligularis*

P. ligularis is one of the *Passiflora* species least studied in terms of phenolic composition. **Figure 2A** shows the chromatogram of *P. ligularis* peel extract obtained by PHWE (see **Table 2**). The main type of phenolic compounds found on the extracts were flavonoids. For instance,

peak 11 showed a molecular ion at m/z 691.2608 $[M-H]^-$ and fragment ions at m/z 631 $[M-60-H]^-$ that correspond to the loss of sugar (myricetin-3-*O*-(6"-galloyl)-galactoside), and fragment ions at m/z 317, 335 and 273 suggesting that this compound could be the flavonol myricetin-3-*O*-(6"-galloyl)-glycoside according to Simirgiotis et al. (2013). Due to absences of a fragment ion at m/z 479 produced by the loss of galloyl group, it is not possible to completely identify this peak. Additionally, the flavonols quercetin-glucoside (**peak 6**, t_R= 26.3 min) and quercetin 3-*O*-(6"-acetyl-glucoside) (**peak 8**, t_R= 27.9 min) were tentatively identified with $[M-H]^-$ ion at m/z 463.0861 and m/z 505.0976, respectively, and they showed the same fragmentation pattern at m/z 300 and m/z 151 corresponding to their aglycone (quercetin) and to the A^- ring fragment released after RDA (retro-Diels-Alder) fission (Dueñas et al., 2008).

On the other hand, the flavones found in *P. ligularis* extracts were luteolin-glucoside (**peak 7**, t_R= 26.8 min) and luteolin 3-*O*-acetyl-glucoside (**peak 10**, t_R= 30.8 min). Peak 7 showed the molecular ion at m/z 447.0947 $[M-H]^-$, with a fragment at m/z 285 corresponding to the loss of an hexose moiety $[M-162-H]^-$ (Kajdzanoska et al., 2010). Peaks 7 and 10 showed the same fragments at m/z 133 and 112 which are characteristics of luteolin (Li et al., 2016). On the other hand, peak 10 exhibited a molecular ion at m/z 489.1026 $[M-H]^-$ and a fragment ion at m/z 285 which correspond to the loss of an acetyl group and hexose sugar $[M-42-162-H]^-$ (Kajdzanoska et al., 2010).

Moreover, the flavones, apigenin-8-C-glucoside (vitexin) (**peak 9**, t_R= 30.5 min) and apigenin-7-(6"-*O*-acetyl)-glucoside (**peak 12**, t_R= 34.6 min), which gave a $[M-H]^-$ at m/z 431.0981 and 473.1090, respectively, were tentatively identified in *P. ligularis* peel extracts. Both molecule ions produced a main fragment at m/z 269 corresponding to their aglycone apigenin. Also peak 9 exhibited fragments at m/z 311 $[M-120-H]^-$ that correspond to the loss of sugar and peak 12 showed fragments at m/z 413 $[M-60-H]^-$ from a sugar loss and at m/z 311 $[M-162-H]^-$ related

384 to the loss of hexose sugar. These MS patterns have been previously proposed for these
385 flavones (Simirgiotis et al., 2013; Betim Cazarin et al., 2016).
386 The flavanols detected in *P. ligularis* peel extracts were (epi)catechin-glucoside, procyanidin
387 dimer and procyanidin trimer (**peak 2, 4** and **5,** t_R= 3.3, 5.6 and 6.6 min). For peak 2, MS base
388 peak ([M-H]$^-$) of m/z 451.1256 was detected as well as the fragment of m/z 289, which
389 indicated that the monomeric unit is catechin or epicatechin. Both compounds have the same
390 fragmentation pattern due to their isomerity and they could not be distinguished in this specie.
391 **Peaks 4** and **5** presented the molecular ion at m/z 577.1340 and 865.1957 [M-H]$^-$, respectively,
392 and the fragmentation pattern was very similar. For instance, peak 4 showed fragments at m/z
393 407 [M-H-gallic acid (GA, 170 Da)]$^-$ and 289 [flavanol monomer-H]$^-$, while peak 5 displayed
394 MS/MS at m/z 726, 525, 407 [flavanol dimer-H-GA]$^-$ and 289 [flavanol monomer-H]$^-$.
395 Additionally, two different phenolic acids were tentatively identified in *P. ligularis* peel
396 extracts. The carboxylic acid, orsellinic acid-2-*O*-β-glucoside (**peak 1**, t_R= 2.7 min) presented
397 the molecular ion with m/z 329.0876 [M-H]$^-$ and MS/MS yielded ions at m/z 167 that
398 corresponded to orsellinic acid because of the loss of hexose [M-162-H]$^-$, and at m/z 123 due
399 to the orsellinic acid decarboxilation [M-162-44-H]$^-$ (Musharraf et al., 2015). However, **peak**
400 **3** (t_R= 3.7 min) displayed a [M-H]$^-$ ion at m/z 359.0997 with fragment ions at m/z 197 [M-162-
401 H]$^-$ due to the loss of hexose. This ion (m/z 197) yielded characteristic fragment ions at m/z
402 182 and 153 which are specific of syringic acid fragmentation. This compound was tentatively
403 identified as glucosyringic acid.
404 To our knowledge, this is the first time that phenolic compounds from *P. ligularis* peels are
405 described.
406 **3.3.2. *Passiflora edulis***
407 *P. edulis* is one of the most studied types of Passifloraceae. The majority of studies are related
408 to phenolic compounds analysis by HPLC-DAD and HPLC-DAD-ESI/MS/MS from leaves,

409 peel flour and peels (Zucolotto et al., 2012; Betim Cazarim et al., 2016; Gomes et al., 2017).
410 Regarding phenolic compounds from *P. edulis* peels, several compounds have been identified
411 by HPLC-DAD as major constituents such as quercetin-3-*O*-glucoside and edulilic acid (a
412 novel cyclic acid found in this *Passiflora* specie) and in lower quantities catechin, epicatechin,
413 kaempferol 3-*O*-glucoside, kaempferol, luteolin-8-C-neohesperidoside, luteolin-8-C-
414 digitoxoside, protocatechuic acid, quercetin and prunasin (Zibadi et al., 2007). To our
415 knowledge, there are no reports on phenolic composition of *P. edulis* peel extracts obtained by
416 PHWE including different polyphenolic families.
417 In accordance with Zibadi et al. (2007) in this investigation the predominant peak (**peak 6**, t_R
418 =17.8 min) in *P. edulis* chromatogram (**Figure 2B**) which presented its maximum absorption
419 at 280, 350 and 520 nm on the UV spectrum was tentatively identified as cyanidin glucoside.
420 This anthocyanin presented the molecular ion at m/z 449.1073 $[M+H]^+$ and the main fragment
421 at m/z 287 as a result of the loss of a hexose sugar molecule $[M-162+H]^+$ and it corresponds to
422 its aglycone (see **Figure 3A**). Possibly, this compound could be responsible for the red color
423 of *P. edulis* peel.
424 The group of flavonoids namely flavones were detected as majority group in *P. edulis* extracts.
425 **Peaks 8** (t_R =24.8 min), **10** (t_R =27.0 min) and **11** (t_R =28.0 min) were tentatively identified as
426 luteolin-rhamnosyl-glucoside, luteolin-glucoside and luteolin-3-glucosyl-rhamnoside,
427 respectively. Luteolin-rhamnosyl-glucoside showed the same molecular ion as luteolin-3-
428 glucosyl-rhamnoside at m/z 593.1464 $[M-H]^-$ (**Figure 3B**). However, the fragmentation pattern
429 from both compounds was different, while luteolin-rhamnosyl-glucoside presented fragments
430 at m/z 473 $[M-120-H]^-$ and correspond to the loss of C-glucosyl moiety, 429 $[M-120-CO^2-H]^-$
431 , 357, 327, 309 and 285 [M-162-146-H], luteolin-3-glucosyl-rhamnoside had fragments at m/z
432 447 $[M-146-H]^-$, 429 $[M-146-H_2O-H]^-$, and 285 $[M-146-162-H]^-$. The latter signal corresponds
433 to the aglycone moiety which indicates a loss of the rhamnose (146) and glucose (162) in

agreement with previously reported data (Ibrahim et al., 2015). Furthermore, luteolin-glucoside (peak 10) displayed $[M-H]^-$ ion at m/z 447.0914 and the MS/MS spectra of this ion showed the main fragment at m/z 285 that corresponded to the product ion of the aglycone because of the loss of hexose sugar $[M-162-H]^-$. The latter three phenolic compounds had the same fragments at m/z 133 and 112, according to the literature, these fragments are characteristic of luteolin (Coutinho et al., 2016).

On the other hand, **peaks 7** (t_R =23.3 min), **9** (t_R =26.1 min) and **12** (t_R =28.1 min) were tentatively identified as flavonols quercetin 3-*O*-(6"malonyl-glucoside)-7-*O*-glucoside, quercetin glucoside (see **Figure 3C**) and quercetin 3-*O*-(6"acetyl-glucoside), respectively (**Figure 2B** and **Table 2**). **Peak 7** showed a $[M-H]^-$ ion at m/z 711.2140 and base peak product ions at m/z 505 $[M-120-86-H]^-$ that correspond to release of sugar and malonyl (86 Da) moieties. Besides, similar daughter fragment ion was detected at m/z 301 related to quercetin structure. **Peak 9** presented MS base peak ($[M-H]^-$) of 463.0879 and it produced a clear fragment corresponding to its aglycone at m/z 301 $[M-162-H]^-$. **Peak 12** showed the molecular ion at m/z 505.0963. This molecule ion produced the main fragments at m/z 463 $[M-42-H]^-$ and 301 $[M-42-162-H]^-$, which were caused by loss of acetyl moiety (42 Da) and the hexoside sugar (162 Da). These fragmentation patterns have been previously reported in the literature (Kajdzanoska et al., 2010).

Other group of flavonoids that could be identified in *P. edulis* were the flavanols. For instance, **peak 3** (t_R =6.7 min) and **5** (t_R =16.4 min) (**Figure 2B** and **Table 2**) were tentatively identified as catechin (see **Figure 3B**) and epicatechin, respectively. Both compounds are isomers and they showed the same molecular ion at m/z 289 $[M-H]^-$. However, they could be distinguished by their retention times found in the literature (Plaza et al., 2017). These molecule ions (m/z 289) produced a main fragment at m/z 137 [M-H-galloyl group (G, 152 Da)]$^-$, 125 formed by A ring cleavage, and 109 $[M-H-G-CO]^-$, thus clearly indicate presence of these monomers.

Moreover, **peak 1** (t_R=3.4) and **peak 4** (t_R=8.6) were tentatively identified as (epi)catechin glucoside isomers since they presented a $[M-H]^-$ at m/z 451.1239 and MS/MS yielding ions m/z 289, 137, 125 and 109. The fragment at m/z 289 is the result of the loss of a hexose sugar $[M-162-H]^-$ and other fragments obtained at m/z 137, 125 and 109 have been described above for (epi)catechin and these ions could be observed in fragmentation patterns for all flavanols (see **Table 2**).

The mass spectra of **peak 13** (t_R =29.0 min) indicated mass spectra similar to phloretin glucoside by the molecular ion $[M-H]^-$ m/z 435.1304 and the main fragments at m/z 273 as a result of the loss of hexose sugar $[M-162-H]^-$ and 167 (**Figure 3F**). It agrees with the MS data reported in the literature for this dihydrochalcone (Bystrom et al., 2008).

Peak 14 (t_R =32.8 min) was tentatively identified as artemitin, also known as 5-hydroxy-3,3',4',6,7-pentamethoxy-flavone; it is considered to be a flavonoid lipid molecule. Hydroxybenzoic acid was also found in *P. edulis* extracts. For instance, protocatechualdehyde acid (**peak 2**, t_R =4.6 min) which presented the molecular ion at $[M-H]^-$ m/z 137.0245 and was tentatively identified.

In accordance with Zucolotto et al. (2011), flavones such as orientin or vitexin have not been detected in these *P. edulis* peel extracts. To our knowledge, this is the first time that phenolic compounds such as quercetin 3-*O*-(6" malonyl-glucoside)-7-*O*-glucoside, quercetin 3-*O*-(6"-acetyl-glucoside), luteolin-rhamnosyl-glucoside, luteolin-glucoside, protocatechualdehyde acid and phloretin glucoside are identified in *P. edulis*.

3.3.3. *P. edulis* flavicarpa

In agreement with literature, the analysis of *P. edulis* flavicarpa peel extracts by HPLC-DAD-QTOF-MS and MS/MS (**Figure 2C** and **Table 2**) showed that flavones were the main phenolic group present in the extracts (Zeraik et 2016). The peak with the highest area was tentatively identified as luteolin-6-C-glucoside ((iso)orientin) (**peak 4**, t_R= 23.8 min) which showed a

484 molecular ion at m/z 447.0933 $[M-H]^-$ and fragment ions at m/z 357 $[M-90-H]^-$, 327 $[M-120-H]^-$, 297 $[M-90-60-H]^-$ which corresponded to the loss of hexose sugar moiety (**Table 2**). This fragmentation pattern was previously reported by Betim Cazarin et al. (2016) and Zeraik et al. (2010) for isoorientin from *P. edulis* peel and *P. edulis* flavicarpa pulp, respectively. **Peak 1** (t_R= 19.8 min) exhibited a molecular ion at m/z 609.1457 $[M-H]^-$ and fragment signals at m/z 489 $[M-120-H]^-$, 429 $[M-120-60-H]^-$, 399 $[M-120-90-H]^-$ and 369 $[M-120-120-H]^-$, indicating the loss of two glucose residues. This compound was tentatively characterized as 6,8-di-C-glycosyl luteolin, commonly known as luteolin-(7-*O*-glucopyranosil)-8-C-glucoside (lucenin). This flavone has been identified in different Passifloras species such as *P. edulis* peel (Beetim Cazarin et al., 2016), *P. mollissima* peel (Simirgiotis et al., 2013) or *P. edulis* flavicarpa leaves (Simirgiotis et al., 2013). **Peak 5** (t_R = 24.9 min) had the same retention time, molecular ion and fragmentation pattern as peak 8 in *P. edulis*. Therefore, peak 5 was tentatively identified as luteolin-rhamnosyl-glucoside (**Figure 3D**). Besides, **peak 8** (t_R = 24.9 min) showed the same molecular ion (m/z 593.1504) as peak 5. However, the fragmentation pattern was different (see **Table 2**). The fragmentation pattern of peak 8 yielded the main fragment at m/z 285 which suggested the loss of the disaccharide rutinose $[M-308-H]^-$ composed of rhamnose and glucose (Schumbert et al., 2010) and it was tentatively identified as luteolin-rutinoside. On the other hand, **peak 9** and **peak 11** (t_R= 29.9 and 32.2 min, respectively) presented the same molecular ion m/z 431 $[M-H]^-$ and the fragmentation pattern was identical (see **Table 2**). These ions showed fragments at m/z 357 $[M-74-H]^-$ and 327 $[M-104-H]^-$ which are characteristic of a C-linked hexose deoxy sugar such as fucose, as well as at m/z 285 corresponding to the aglycon moiety that indicated a loss of fucose (Benayad et al., 2014). These peaks were both tentatively identified as 6-C-fucosylluteolin which has been previously described in *P. edulis* flavicarpa leaves (Mareck et al., 1991). **Peak 3** (t_R = 21.8 min) revealed the existence of a molecular ion $[M-H]^-$ at m/z 579.1334. This molecule ion produced fragments at m/z 489 $[M-90-H]^-$, 459 [M-

120-H]$^-$, 399 [M-120-60-H]$^-$, 369 [M-120-60-90-H]$^-$. These neutral losses are characteristic of a glucose and pentose residues as previously reported (Benayad et al., 2014). This compound was identified as luteolin-(6-C-pentosyl)-8-C-glucoside.

Other flavones found in *P. edulis* flavicarpa were derived of apigenin. For instance, **peak 7** (t_R= 26.2 min) was tentatively identified as apigenin-8-C-β-D-glucoside (vitexin) because it had a base peak at m/z 431.0977 [M-H]$^-$ and showed fragments at m/z 341 [M-90-H]$^-$, 311 [M-120-H]$^-$, 283 and 269 [M-162-H]$^-$ (**Table 2** and **Figure 2C**) (Betim Cazarin et al., 2016). These neutral losses are characteristic of a hexose sugar (glucose) and the latter ion corresponds to its aglycone (apigenin). **Peak 2** (t_R= 21.7 min) was tentatively identified as apigenin dihexoside considering its molecular ion at m/z 593.1504 [M-H]$^-$ and the presence of characteristic fragments of the loss of two hexosyl moieties (m/z at 473 [M-120-H]$^-$, 431 [M-162-H]$^-$, and 353 [M-120-120-H]$^-$). Besides, apigenin rhamnosyl-glucoside (**peak 10**, t_R = 31.3 min) was tentatively identified with a molecular ion at m/z 577.1404 [M-H]$^-$ and fragment ions at m/z 473 [M-104-H]$^-$, 413 [M-164-H]$^-$, 357 [M-104-116-H]$^-$ and 327 [M-104-146-H]$^-$ which are characteristic of a C-linked rhamnosyl and C-linked glucosyl (Benayad et al., 2014).

Peak 6 (t_R= 25.2 min) was the second compound with the highest peak area in *P. edulis* flavicarpa peel extracts. This compound was identified as the phenolic acid ellagic acid which presented MS base peak at m/z 300.9983 [M-H]$^-$ and this compound was identified as free ellagic acid confirmed by characteristic ions at m/z 130, 229 and 283 upon dissociation (see **Figure 3A**).

In this work eleven phenolic compounds were identified in different families from *P. edulis* flavicarpa peel extracts obtained by PHWE, from which four phenolic compounds have been identified for the first time in *P. edulis* flavicarpa (ellagic acid, 6-C-fucosylluteolin isomer, luteolin-rhamnosyl-glucoside and apigenin rhamnosyl-glucoside rutinoside).

3.3.4. P. mollissima

P. mollissima is one of the least studied species of Passiflora with regards to phenolic compounds. Previous studies revealed the presence of flavones in *P. mollissima* pericarps such as isoorientin, orientin and isovitexin (Zucolotto et al., 2012). On the other hand, Simirgiotis et al. (2013) identified phenolic compounds in peel extracts obtained by UAE and analyzed by HPLC-ESI-MS-MS (Simirgiotis et al., 2013). Nevertheless, the information on phenolic compounds from *P. mollissima* peels are very limited and there are no reports of an exhaustive identification of phenolic compounds from *P. mollissima* peel extracts obtained by PHWE (Simirgiotis et al., 2013).

Peak areas showed that the majority compounds in *P. mollissima* extracts belong to flavone group (see **Table S1**). With regard to flavones, the peak with the highest area was **peak 18** (t_R= 26.4 min) with a molecular ion at m/z 593.1526 $[M-H]^-$ that exhibited the same molecular ion as **peak 11** (t_R= 24.3 min) and **peak 14** (t_R= 25.5 min). However, the fragmentation pattern of these three compounds was different. While peaks 11 and 14 were tentatively identified as luteolin-rhamnosyl-glucoside derivatives because they showed the same molecular ion and fragmentation pattern as peak 9 in *P. edulis* and peak 5 in *P. edulis* flavicarpa; peak 18 was tentatively characterized as luteolin-rutinoside having the same fragment profile a peak 8 of *P. edulis* flavicarpa. In agreement with Simirgiotis et al. (2013), (iso)orientin (**peak 10**, t_R= 23.8 min) was detected with a molecular ion at m/z 447.0918 $[M-H]^-$. This compound was determined in *P. edulis* flavicarpa (peak 4) with the same spectra profile (see **Table 2**).

Peak 16 (t_R= 25.7 min) was tentatively identified as luteolin-7-gentibioside which presented the same molecular ion as **peak 15** (t_R= 25.6 min) but the fragmentation pattern was different. Peak 16 exhibited a molecular ion at m/z 609.1462 $[M-H]^-$ and yielded the main fragment at m/z 285 which suggested the loss of the disaccharide formed for two hexosyl moieties [M-162-162-H]$^-$. However, peak 15 was identified as luteolin dihexoside due to the fragment ions at m/z 447 $[M-162-H]^-$, 327 $[M-162-120-H]^-$ corresponding to the loss of two hexosyl moieties

559 and the characteristic ion at m/z 112 of luteolin molecule. **Peak 9** (t_R= 23.0 min) was tentatively
560 identified as lucenin which showed fragment ions at m/z 489 $[M-120-H]^-$, 447 $[M-162-H]^-$, 357
561 $[M-162-90-H]^-$, 327 $[M-162-120-H]^-$ and 285 $[M-162-162-H]^-$ which indicated the loss of two
562 hexosyl moieties.
563 On the other hand, **peak 8** (t_R= 21.9 min) was tentatively identified as isoorientin-7-rutinoside
564 because its $[M-H]^-$ ion at m/z 755.2028 and its main fragment ions at 635 $[M-120-H]^-$, 593 [M-
565 162-H]$^-$, 473 $[M-162-120-H]^-$, 357 $[M-162-120-116-H]^-$, 327 $[M-162-120-146-H]^-$ and 285
566 $[M-162-162-146-H]^-$ describing the loss of two glucosyl and one rhamnosyl moieties.
567 Additionally, diosmetin rutinoside (**peak 20**, t_R= 27.8 min) was tentatively identified with a
568 $[M-H]^-$ ion at m/z 607.1676 and fragment ions at m/z 443 $[M-146-18-H]^-$ and 383 [M-146-18-
569 60-H]$^-$ that correspond to the profile fragmentation of diosmetin molecule, fragment at m/z 341
570 is related to the losses of acetyl residues (-42 Da), and characteristic fragments of diosmetin
571 molecule such as m/z 299 and m/z 269 were detected which correspond to rutinose moiety
572 (Roowi et al., 2011).
573 Other flavones found in *P. mollissima* were apigenin derivatives. For instance, **peak 19** (t_R=
574 26.6 min) was tentatively identified as apigenin-8-C-glucoside (vitexin) with a $[M-H]^-$ ion at
575 m/z 431.0993 and the main fragment ion at m/z 311. This fragment is considered to be
576 originated from the cross-ring cleavage of the glucose residue (120 Da). This compound has
577 been found in *P. edulis* flavicarpa peel extracts (peak 7) and it has been previously reported in
578 *P. mollissima* peels (Simirgiotis et al., 2013). Furthermore, vitexin-2"-rhamnoside (**peak 17**,
579 t_R= 26.0 min) could be identified since it showed a $[M-H]^-$ ion at m/z 577.1577 and MS/MS
580 yielding ions at m/z 457 $[M-120-H]^-$, 413 $[M-164-H]^-$, 341 $[M-120-116-H]^-$, 293[M-146-18-
581 120-H]$^-$, 283 and 269 $[M-162-146-H]^-$, which are characteristic of a C-linked rhamnosyl and
582 C-linked glucosyl (Benayad et al., 2014). On the other hand, **peak 7** (t_R= 21.5 min) was
583 tentatively identified as apigenin 7-*O*-neohesperidoside 4'-glucoside (rhoifolin 4-glucoside). It

584 presented a molecular ion at m/z 739.2080 and MS/MS yielding ions at m/z 577 [M-162-H]$^-$,
585 457 [M-162-120-H]$^-$, 413 [M-162-146-18-H]$^-$, 311 [M-162-120-146-H]$^-$, 293 [M-162-120-
586 146-18-H]$^-$ and 269 [M-162-308-H]$^-$. These fragments indicated the loss of two hexoses and
587 one dehydroxyhexose moieties.
588 Moreover, two flavonols were detected in *P. mollissima* peel extracts. For instance, myricetin
589 (**peak 6**, t_R= 16.7 min) was tentatively identified because it showed a molecular ion at m/z
590 317.1251 and the main fragment ion at m/z 155 upon dissociation. The other flavonol found in
591 *P. mollissima* was quercetin rutinoside (**peak 12**, t_R= 24.4 min) with a molecular ion at
592 609.1464 and MS/MS yielding ions at m/z 300 which suggested the loss of the disaccharide
593 rutinose.
594 As well as *P. ligularis* and *P. edulis, P. mollissima* presented flavanols as (epi)catechin
595 glucoside (**peak 2**, t_R= 3.3 min), procyanidin dimer (**peak 3**, t_R= 5.7 min), catechin (**peak 4**,
596 t_R= 6.4 min) (see **Figure 3D**), (epi)catechin glucoside derivative (**peak 5**, t_R= 8.2 min) which
597 presented the same retention time, molecular ion and fragmentation pattern than these
598 compounds in the other *Passiflora* species. However, *P. mollissima* showed also (epi)catechin-
599 (epi)gallocatechin (**peak 1**, t_R= 3.0 min). This flavanol was tentatively identified because it had
600 a [M-H]$^-$ ion at 593.1309 and MS/MS fragments at m/z 467 [M-126-H]$^-$, 441 [M-152-H]$^-$, 423
601 [M-152-18-H]$^-$, 407, 305 and 289. Loss of 126 Da indicates that A ring of the upper unit has a
602 1,3,5-trihydoxybenzene structure (Gu et al., 2003), loss of 152 Da was assigned to the loss of
603 one galloyl group, the ion at m/z 305 is the (epi)gallocatechin group while the ion at m/z 289
604 means the (epi)catechin group. All these fragments were characteristic of an (epi)catechin-
605 (epi)gallocatechin (Tala et al., 2013).
606 Ellagic acid (**peak 13**, t_R= 25.1 min) was also found in *P. mollissima* as well as in *P. edulis*
607 flavicarpa, and the MS spectra is described in Section 3.3.3.

608 This is the first time that ellagic acid, (epi)catechin-(epi)gallocatechin, procyanidin dimer,
609 catechin, (epi)catechin glucoside and its isomer, quercetin rutinoside, apigenin-7-*O*-
610 neohesperidoside and luteolin-7-gentibioside have been identified in *P. mollissima* peel.

611 **3.4 Relationship between the individual phenolic compounds, and the *in vitro* antioxidant**
612 **capacity, the total phenolic content and the intracellular ROS scavenging capacity**
613 **present in of the PHWE extracts of peels from different *Passiflora* species**

614 The phenolic compounds identified in different *Passiflora* species belong to different phenolic
615 groups such as phenolic acids, flavanols, flavonols, flavones, chalcones or anthocyanidins,
616 among others. In order to know the contribution of main groups of phenolic compounds to the
617 antioxidant capacity in the different *Passiflora* species, a statistical study on the possible
618 correlation between the total antioxidant capacity measured by DPPH, TEAC, FC and
619 intracellular ROS scavenging assays and the total areas of the different phenolics groups
620 obtained by HPLC-DAD was carried out. The intracellular ROS scavenging assay were not
621 directly correlated with any group and any individual phenolic compounds. **Table S1** shows
622 the total peak areas of groups of phenolic compounds determined by HPLC-DAD at 280 nm to
623 understand their contribution to the antioxidant capacity. Our results indicated a significant
624 correlation ($p \leq 0.05$) with different groups of phenolic compounds. Regarding DPPH assay, a
625 positive correlation ($r > 0.90$) was shown for phenolic acids. However, TEAC assay displayed
626 a positive correlation ($r > 0.90$) with chalcones. Additionally, a correlation between groups of
627 phenolic compounds was carried out with FC assay. Results showed a poor correlation between
628 total phenolic compounds and the different phenolic groups being the group of flavones, the
629 one which presented a greater correlation ($r = 0.75$).

630 On the other hand, the contribution of individual phenolic compounds to the total antioxidant
631 capacity was achieved. Ellagic acid and the flavones apigenin dihexoside and apigenin
632 rhamnosyl-glucoside showed a positive correlation ($r > 0.80$) with the total antioxidant capacity

633 measured by DPPH assay, while in TEAC assay were (epi)catechin glucoside and phloretin
634 glucoside. Nevertheless, apigenin dihexoside identified in *P. edulis* flavicarpa was the main
635 phenolic compound which contributed to the antioxidant power employing DPPH assay with
636 a correlation of 0.92. Phloretin glucoside was the most important contributor when TEAC assay
637 was used with a correlation of 0.90 which was found in *P. edulis*. FC assay presented positive
638 correlation with quercetin 3-*O*-(6"-acetyl-glucoside) and luteolin glucoside, being luteolin
639 glucoside the most important contributor with a correlation of 0.94 which was found in *P.*
640 *ligularis* and *P. edulis*.
641 Previous studies have shown that phenolic compounds apigenin dihexoside, phloretin
642 glucoside and quercetin 3-*O*-(6"-acetyl glucoside) are powerful antioxidants (Lee et al., 2002;
643 Smiljkovic et al., 2017; Xu et al., 2016). In this sense, apigenin and their derivatives have
644 exhibited significant antioxidant effect (Smiljkovic et al., 2017). Phloretin glucoside which is
645 present mainly in apples and apple seeds has been recognized as a potential antioxidant, and it
646 has been suggested that its antioxidant capacity could inhibit lipid peroxidation (Xu et al.,
647 2016). Besides, quercetin 3-*O*-(6"-acetyl glucoside) from leaves of *Carthamus tinctorius* has
648 shown antioxidant effects in rats (Lee et al. 2002).
649

650 **4. CONCLUSIONS**

651 HPLC-DAD-QTOF-MS and MS/MS analysis of peel extracts obtained by PHWE from four
652 *Passiflora* species revealed that this residue from the food industry could be an interesting
653 source of antioxidant phenolic compounds with a complex qualitative composition increasing
654 their added value. In fact, *P. mollissima* and *P. edulis* peel extracts presented higher antioxidant
655 capacity and TPC than *P. ligularis* and *P edulis* flavicarpa. Also, *P. mollissima* and *P. edulis*
656 peel extracts showed a high reduction on intracellular ROS production being a very promising
657 antioxidant extracts. A tentative structural elucidation of 57 phenolic compounds from these

extracts was achieved by the developed HPLC-DAD-QTOF/MS method. They belong to different phenolic groups such as phenolic acid, flavanols, flavonols, flavones, hydroxybenzoic acids, chalcones and anthocyanidins being this work the first time that many of the phenolics have been described in these *Passiflora* species peels. The main classes of polyphenols found in the PHWE extracts that may contribute to their total antioxidant capacity were phenolic acids, flavones and chalcones. The results obtained in this work demonstrate that passion fruit peels can constitute a sustainable source of antioxidant compounds whose revalorization could allow the reduction of the environmental impact of these residues by increasing also the possibility to obtain economic benefits by food companies.

CONFLICTS OF INTEREST

The authors have declared no conflict of interest.

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FIGURE CAPTIONS

Figure 1. Effect of *Passiflora* species peel extracts at different concentrations (25-1000 μg/mL) on cell viability (A) and intracellular ROS generation in HeLa cells. (B) Different letters denote statistically significant differences between all treatments ($p \leq 0.05$)

Figure 2. Chromatogram of phenolic compounds of freeze-dried peel from four *Passiflora* species at 280 nm: (A) *P. ligularis* (B) *P. edulis*, (C) *P. edulis* flavicarpa and (D) *P. mollissima*. For peak identification, see Table 2.

Figure 3. MS/MS spectra of the phenolic compounds found in PHWE extracts from *Passiflora* species peels; (A) cyanidin glucoside, (B) luteolin rhamnosyl glucoside, (C) quercetin glucoside, (D) catechin, (E) phloretin glucoside, (F) apigenin rhamnosyl glucoside and (G) ellagic acid.

Table 1. Antioxidant capacity of the extracts by DPPH assay expressed as EC_{50} (µg/mL extract), TEAC assay expressed as mmol trolox/g of dried extract and TPC assay expressed as mg GAE/g of dried extract.

***Passiflora* Specie**	**DPPH**	**TEAC**	**TPC**
P. ligularis	298.57 ± 18.31^{b}	0.05 ± 0.01^{b}	5.08 ± 0.48^{d}
P. edulis	32.93 ± 2.88^{a}	2.01 ± 0.01^{a}	24.96 ± 2.00^{b}
P. edulis flavicarpa	718.91 ± 40.55^{c}	0.08 ± 0.01^{b}	8.34 ± 0.83^{c}
P. mollisima	10.56 ± 0.80^{a}	2.24 ± 0.15^{a}	30.19 ± 3.00^{a}

[a,b,c] Superscript letters show the significant differences among *Passiflora* species from the same assay ($p \leq 0.05$). Letter *a* shows the most effective extract decreasing its effectiveness when letters progress in the alphabet. Extracts with the same letter show no statistically significant differences.

Table 2. Mass spectra data and maximum absorption of the phenolic compounds identified in *Passiflora* species peel extracts by HPLC-DAD-ESI-QTOF/MS and MS/MS.

Passiflora specie	ID	RT (min)	Compound identified	Experimental m/z [M-H]-	Molecular formula	Error (ppm)	Main fragments detected m/z	UV-vis (nm)	Score
P. ligularis	1	2.7	Orsellinic acid-2-*O*-β -glucoside	329.0876	$C_{14}H_{18}O_9$	1.1	167.0347, 123.0447, 108.0213	250, 260, 290, 300	98.21
	2	3.3	(epi)catechin glucoside	451.1256	$C_{21}H_{24}O_{11}$	1.5	367.8731, 289.0711	260, 270	94.60
	3	3.7	Glucosyringic acid	359.0997	$C_{15}H_{20}O_{10}$	3.7	197.0459, 182.0221, 153.0565	215, 260	94.15
	4	5.6	Procyanidin dimer	577.1340	$C_{30}H_{26}O_{12}$	2.1	407.0727, 367.0857, 289.0715	270, 280	97.24
	5	6.6	Procyanidin trimer	865.1957	$C_{45}H_{38}O_{18}$	1.2	726.3690, 525.0756, 407.0779, 289.0721	230, 370	95.95
	6	26.3	Quercetin-glucoside	463.0861	$C_{21}H_{20}O_{12}$	1.5	300.0256, 151.0009	230, 360	95.76
	7	26.8	Luteolin-glucoside	447.0947	$C_{21}H_{20}O_{11}$	2.6	285.0384, 174.9501, 133.0266, 112.9860	230, 370	95.50
	8	27.9	Quercetin 3-*O*-(6"-acetyl-glucoside)	505.0976	$C_{23}H_{22}O_{13}$	2.9	300.0269, 151.0051	230, 360	88.52
	9	30.5	Apigenin-8-C-glucoside (Vitexin)	431.0981	$C_{21}H_{20}O_{10}$	1.2	311.0555, 269.0462	270, 350	93.36
	10	30.8	Luteolin 3-*O*-acetyl-glucoside	489.1026	$C_{23}H_{21}O_{12}$	2.3	285.0380, 179.8099, 133.0328, 112.9874	230, 330, 350, 360	96.49
	11	31.2	Myricetin-3-*O*-(6"-galloyl)-glycoside	691.2608	$C_{34}H_{44}O_{15}$	0.1	631.1510, 335.1332, 317.1148, 273.1257	230, 250, 360	95.24
	12	34.6	Apigenin 7-(6”-*O*-acetyl)-glucoside	473.1090	$C_{23}H_{22}O_{11}$	0.1	413.0823, 373.2262, 311.0534, 269.0437	270, 340	98.57
P. edulis	1	3.4	(epi)catechin glucoside isomer	451.1239	$C_{21}H_{24}O_{11}$	-2.6	289.0717, 137.0231, 125.0236, 109.0291	260, 280	90.40
	2	4.6	Protocatechualdehyde acid	137.0245	$C_7H_6O_3$	0.1	108.0217	260, 270, 290, 300, 310	99.21
	3	6.7	Catechin	289.0711	$C_{15}H_{14}O_6$	2.5	137.0231, 125.0237, 109.0290	260, 280	97.38
	4	8.6	(epi)catechin glucoside isomer	451.1244	$C_{21}H_{24}O_{11}$	1.3	289.0732, 137.0269, 125.0267, 109.0262	270, 290	89.99
	5	16.3	Epicatechin	289.0713	$C_{15}H_{14}O_6$	2.8	137.0198, 125.0202, 109.0248	225, 230, 280	93.97
	6	17.8	Cyanidin glucoside	449.1073 $[M+H]^+$	$C_{21}H_{20}O_{11}$	-0.7	287.0553	280, 510, 520	99.23

	7	23.3	Quercetin 3-*O*-(6"malonyl-glucoside)-7-*O*-glucoside	711.2140	$C_{32}H_{40}O_{18}$	0.8	505.1892, 390.0972, 302.1117, 301.1020, 300.0877	240, 260, 270, 310, 360	98.37
	8	24.8	Luteolin-rhamnosyl-glucoside	593.1464	$C_{27}H_{30}O_{15}$	2.6	473.1018, 429.0816, 449.1017, 357.0604, 327.0499, 309.0393, 285.0361, 133.0294	240, 260, 270, 340, 360	96.95
	9	26.1	Quercetin glucoside	463.0879	$C_{21}H_{20}O_{12}$	-4.1	301.0339	240, 270, 350, 370	91.39
	10	27.0	Luteolin glucoside	447.0914	$C_{21}H_{20}O_{11}$	-2.8	285.0409, 133.0262, 112.9845	240, 280, 360	92.11
	11	28.0	Luteolin-3-glucosyl-rhamnoside	593.1526	$C_{27}H_{30}O_{15}$	-2.2	447.0934, 429.0802, 285.0403, 133.0272	240, 260, 350	97.01
	12	28.1	Quercetin 3-*O*-(6"acetyl-glucoside)	505.0963	$C_{23}H_{22}O_{13}$	-1.5	463. 0875, 301.0355	240, 260, 350	98.00
	13	29.0	Phloretin glucoside	435.1304	$C_{21}H_{24}O_{10}$	-2.5	273.0771, 167.0349	240, 270, 350	94.31
	14	32.8	Artemitin	389.1219 $[M+H]^+$	$C_{20}H_{20}O_8$	4.1	345.1216, 303.1105, 243.0126, 201.0058, 149.0219	240, 270, 340, 360	73.62
***P. edulis* flavicarpa**	1	19.8	Luteolin-(7-*O*-glucopyranosil)-8-C-glucoside (Lucenin)	609.1457	$C_{27}H_{30}O_{16}$	0.7	489.1039, 429.0820, 399.0706, 369.0613, 327.0986	270, 340, 360	99.61
	2	21.6	Apigenin dihexoside	593.1504	$C_{27}H_{30}O_{15}$	1.6	431.0877, 473.1100, 353.0683, 297.0762	225, 230, 270, 350	96.58
	3	21.8	Luteolin –(6-C-pentosyl)-8-C-β-D-glucoside	579.1334	$C_{26}H_{28}O_{15}$	1.8	489.1097, 459.0854, 399.0714, 369.0609, 112.9856	270, 350	84.30
	4	23.8	Luteolin-6-C-glucoside (Orientin/isoorientin)	447.0933	$C_{21}H_{20}O_{11}$	0.3	411.0700, 311.0525, 357.0581, 327.0479, 297.0365	230, 270, 350	98.96
	5	24.9	Luteolin-rhamnosyl-glucoside	593.1495	$C_{27}H_{30}O_{15}$	2.3	473.1080, 431.0823, 327.0492, 251.5180, 196.0677, 112.9856	230, 360	92.23
	6	25.2	Ellagic acid	300.9983	$C_{14}H_6O_8$	2.9	283.9970, 229.0138, 174.9533, 130.9651	228, 229, 252	95.82
	7	26.2	Apigenin-8-C-β-D-glucoside (Vitexin)	431.0977	$C_{21}H_{20}O_{10}$	1.9	341.0698, 311.0543, 283.0635, 269.0627, 263.0635, 174.9537	230, 360	96.40
	8	26.8	Luteolin-rutinoside	593.1504	$C_{27}H_{30}O_{15}$	2.0	383.8356, 328.0432, 285.0373, 112.9856	224, 230, 270, 350	93.84

	9	29.9	6-C-Fucosylluteolin isomer	431.0978	$C_{21}H_{20}O_{10}$	1.2	357.0578, 327.0511, 298.0456, 285.0383, 274.9831, 268.0631	228, 320	98.79
	10	31.3	Apigenin rhamnosyl-glucoside	577.1404	$C_{27}H_{30}O_{14}$	1.2	473.1191, 413.0913, 357.0563, 351.0562, 327.0474,	228, 310, 320, 370	89.50
	11	32.2	6-C-Fucosylluteolin isomer	431.0974	$C_{21}H_{20}O_{10}$	2.7	357.0625, 327.0524, 298.0441, 285.0418, 266.9817	230, 350, 360	94.29
P. mollisima	1	3.0	(epi)catechin-(epi)gallocatechin	593.1309	$C_{27}H_{30}O_{15}$	0.8	467.1035, 441.0759, 423.0877, 407.0791, 339.0878, 305.0533, 289.0710, 245.0800, 177.0191, 151.0392, 125.0237	230, 270, 280	99.42
	2	3.3	(epic)catechin glucoside	451.1246	$C_{21}H_{24}O_{11}$	0.5	289.0712	230, 260	97.21
	3	5.7	Procyanidin dimer	577.1356	$C_{30}H_{26}O_{12}$	-1.3	407.0790, 339.0810, 289.0694, 245.0793, 125.0251	230, 260, 270	97.50
	4	6.4	Catechin	289.0719	$C_{15}H_{14}O_6$	-0.5	245.0808, 203.0722, 179.0363, 137.0222, 109.0289	230, 260	99.41
	5	8.2	(epi)catechin glucoside isomer	451.1235	$C_{21}H_{24}O_{11}$	2.9	289.0692, 245.0816, 205.0457, 123.0445	230, 260, 270	89.15
	6	16.7	Myricetin	317.1251	$C_{14}H_{22}O_8$	-2.6	155.0351	260, 360	97.22
	7	21.5	Apigenin 7-neohesperidoside-4-glucoside	739.2095	$C_{33}H_{40}O_{19}$	1.9	577.1141, 457.1128, 413.0854, 311.0567, 293.0447, 283.0553, 269.0405	220, 230, 330, 360	96.12
	8	21.9	Isoorientin-7-rutinoside	755.2050	$C_{33}H_{40}O_{20}$	-1.1	635.9802, 593.1498, 499.9648, 473.9884, 429.0807, 357.0592, 327.8895, 285.0379	220, 230, 270, 320, 350	98.27
	9	23.0	Luteolin-(7-O-glucopyranosil)-8-C-glucoside (Lucenin)	609.1461	$C_{27}H_{30}O_{16}$	3.0	489.1045, 447.0923, 369.0543, 357.0017, 327.0504, 285.0392	260, 350	97.83
	10	23.8	Luteolin-6-C-glucoside ((iso)orientin)	447.0918	$C_{21}H_{20}O_{11}$	2.9	369.0570, 357.0613, 327.0488, 297.0336, 285.0370	260, 350	94.33
	11	24.3	Luteolin-rhamnosyl-glucoside	593.1508	$C_{27}H_{29}O_{15}$	0.8	473.1053, 429.0827, 327.0487, 309.0387,	230, 270, 350	99.42

						298.0459, 285.0390, 133.0153		
12	24.4	Quercetin rutinoside	609.1464	$C_{34}H_{26}O_{11}$	2.9	300.0266	230, 370	87.89
13	25.1	Ellagic acid	300.9985	$C_{14}H_6O_8$	1.7	283.9944, 257.0077, 229.0125, 185.0231, 130.9650	228, 254	98.63
14	25.5	Luteolin rhamonsyl glucoside	593.1445	$C_{34}H_{26}O_{10}$	1.9	503.1123, 473.1023, 357.0543, 327.0433	270, 360	91.25
15	25.6	Luteolin-dihexoside	609.1471	$C_{27}H_{30}O_{16}$	-1.8	447.0953, 327.0396, 112.9860	230, 330	97.11
16	25.7	Luteolin-7-gentiobioside	609.1462	$C_{27}H_{30}O_{16}$	-0.1	447.0922, 285.0391, 112.9861	230, 330	99.40
17	26.0	Vitexin-2"-rhamnoside	577.1577	$C_{27}H_{30}O_{14}$	-2.6	457.1089, 413.0850, 341.0850, 293.0442, 283.0593, 269.0442	230, 270, 330, 350	95.92
18	26.4	Luteolin-rhamnosyl-glucoside	593.1526	$C_{27}H_{30}O_{15}$	1.7	475.0884, 351.9538, 285.0359, 284.0300, 227.0371, 151.0003, 112.9866	230, 330	90.61
19	26.6	Apigenin-8-C-β-D-glucoside (Vitexin)	431.0993	$C_{21}H_{20}O_{10}$	-2.0	311.0569, 283.0639	230, 270, 330, 350	97.82
20	27.8	Diosmitin rutinoside	607.1676	$C_{28}H_{32}O_{15}$	-1.1	443.0948, 383.0700, 341.0643, 327.0451, 311.0551, 300.0532, 299.0553, 298.0435, 284.0291, 269.0505	230, 270, 350, 360	99.10

Figures

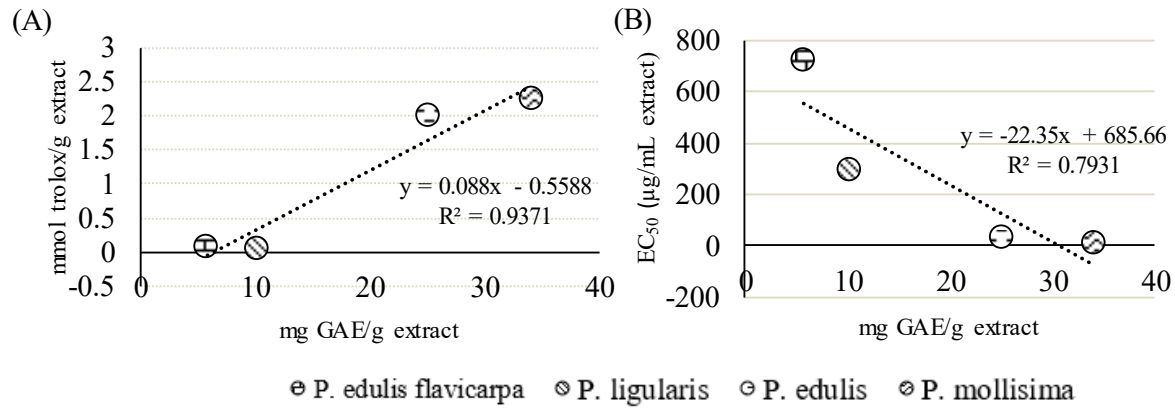

Figure 1.

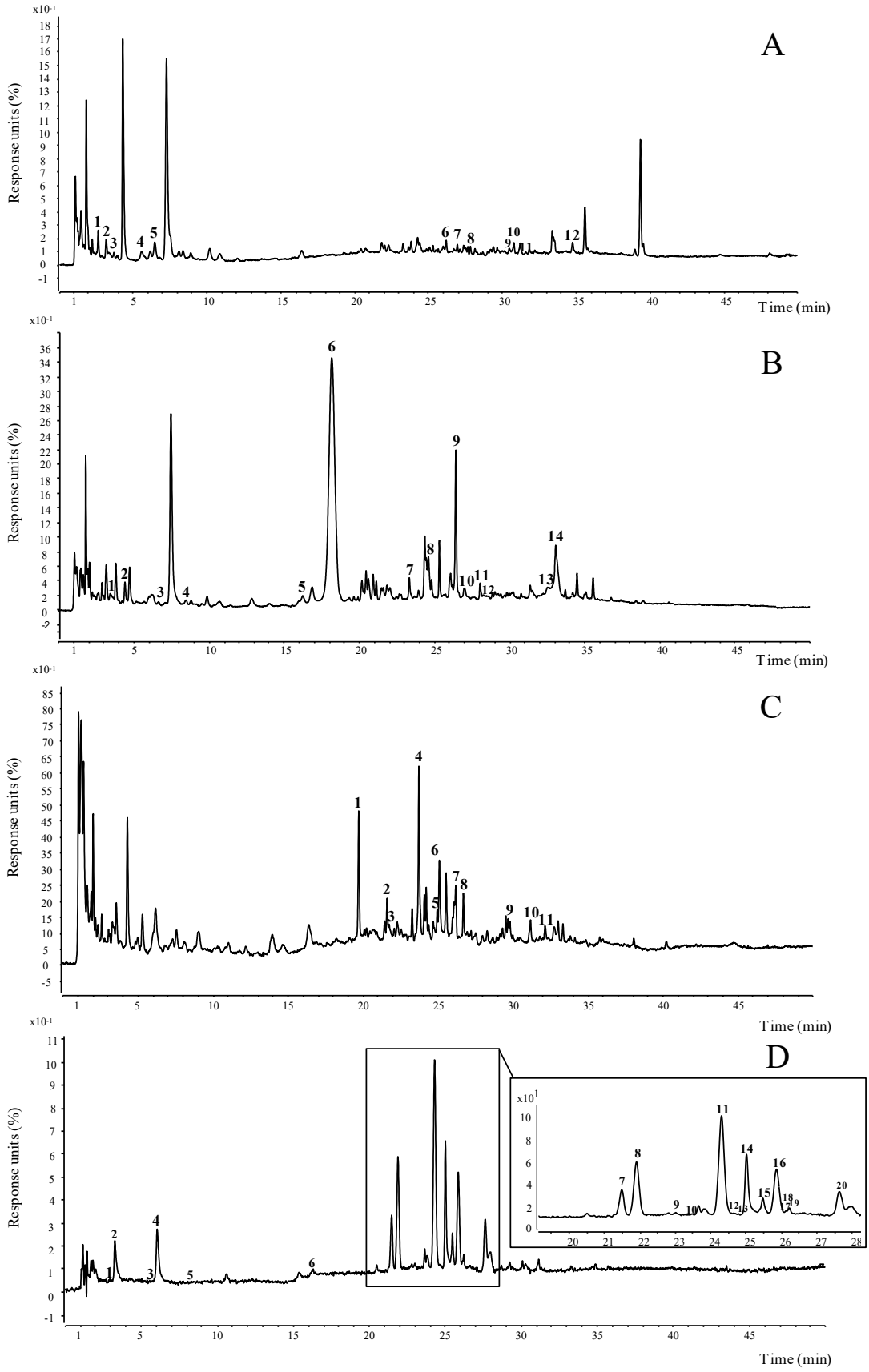

Figure 2.

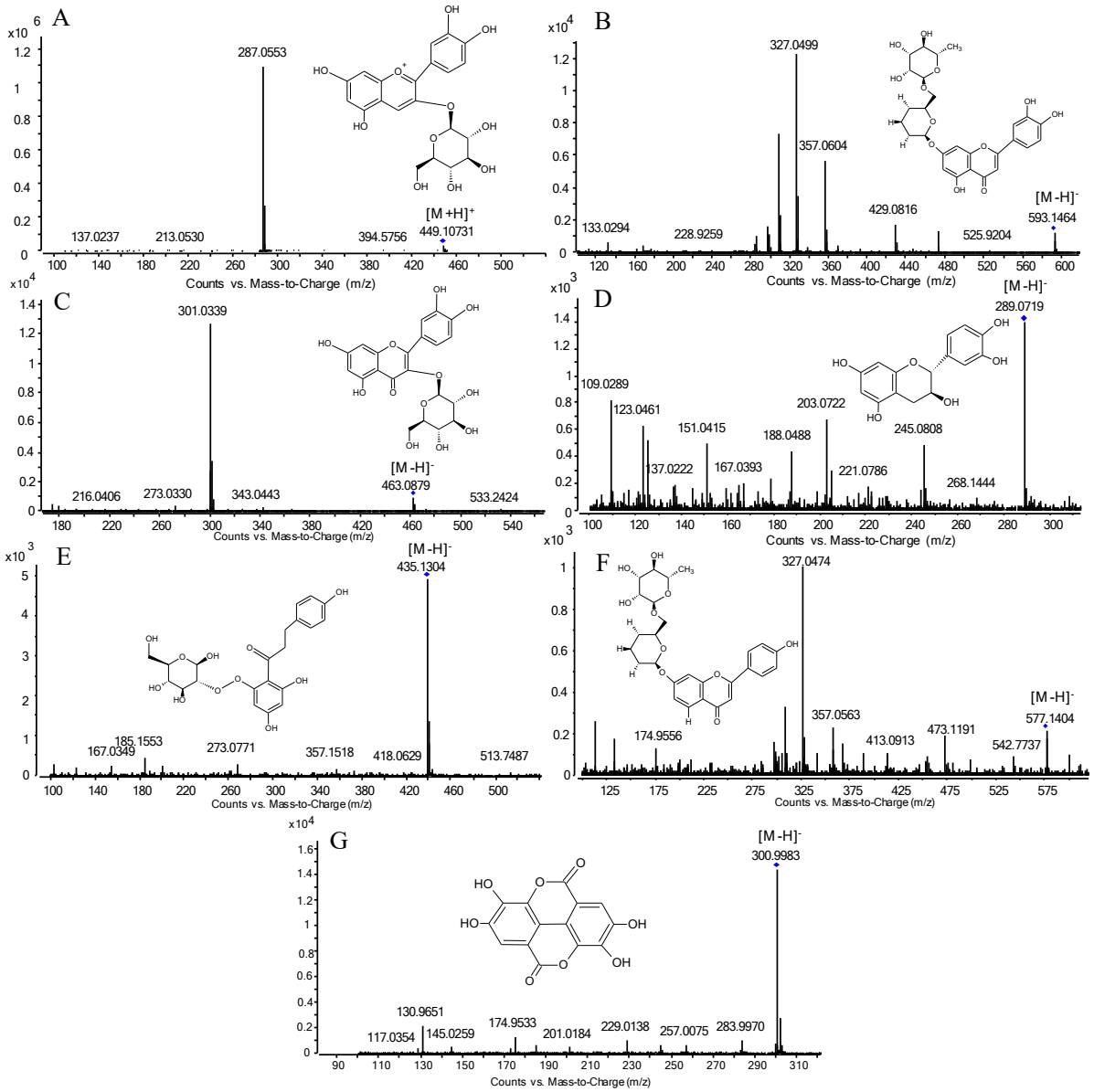
A
287.0553
[M+H]+
449.10731
137.0237
213.0530
394.5756
Counts vs. Mass-to-Charge (m/z)
B
327.0499
357.0604
[M-H]-
593.1464
429.0816
133.0294
228.9259
525.9204
Counts vs. Mass-to-Charge (m/z)
C
301.0339
[M-H]-
463.0879
216.0406
273.0330
343.0443
533.2424
Counts vs. Mass-to-Charge (m/z)
D
[M-H]-
289.0719
109.0289
123.0461
151.0415
203.0722
188.0488
245.0808
137.0222
167.0393
221.0786
268.1444
Counts vs. Mass-to-Charge (m/z)
E
[M-H]-
435.1304
185.1553
167.0349
273.0771
357.1518
418.0629
513.7487
Counts vs. Mass-to-Charge (m/z)
F
327.0474
[M-H]-
577.1404
357.0563
473.1191
174.9556
413.0913
542.7737
Counts vs. Mass-to-Charge (m/z)
G
[M-H]-
300.9983
130.9651
117.0354
145.0259
174.9533
201.0184
229.0138
257.0075
283.9970
Counts vs. Mass-to-Charge (m/z)

Figure 3.