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1 Identification of punicalagin as the bioactive compound behind the antimicrobial

2 activity of pomegranate (*Punica granatum* L.) peels

3

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13

14

15 **ABSTRACT**

16 Extracts from ‘Zhéri’ and ‘Hicaznar’ varieties of pomegranate, *Punica granatum* L., were obtained
17 by subjecting powdered peels to extraction using water, water/ethanol (1:1; v/v), ethanol, acetone
18 and heptane. Using the agar diffusion assay, extracts with water and/or ethanol were shown to
19 display significant antimicrobial activity with diameters of inhibition zones up to 20 mm. Ethanolic
20 extracts, which were the most active, were fractionated using SPE, HPLC and UHPLC, and the
21 active compounds they contain were identified by mass spectrometry. Punicalagin, under its α and
22 β anomeric forms, was identified as the antibacterial compound in pomegranate peel extracts. Both
23 forms were active with MIC values between 0.3 and 1.2 $\mu\text{g}\cdot\text{ml}^{-1}$, and they easily converted from
24 one to the other with an α/β equilibrium ratio of 3/7. Their spectrum of activity targeted 10 out of
25 13 Gram positive and two out of three Gram negative bacteria as well as a yeast strain.

26

27 **Keywords**

28 Pomegranate; *Punica granatum*; punicalagin; antimicrobial activity; peel

29

30

31 **1. Introduction**

32 *Punica granatum* L., commonly known as pomegranate, is native to the Mediterranean region and
33 is cultivated and consumed worldwide today as either fresh fruit, extracted juice, jam, infusion or
34 ingredient in various recipes. It has been used in folk medicine since ancient times and is known for
35 a range of health promoting effects including antioxidant, anti-inflammatory, anti-cancer, anti-
36 diabetic, and antimicrobial properties (Andrade et al., 2019). The most commonly found bioactive
37 molecules are anthocyanins and hydrolysable tannins, namely ellagitannins, gallotannins,
38 flavonoids, lignans, triterpenoids, phytosterols, fatty acids, organic acids and phenolic acids.
39 Pomegranate peels (PgP), the main waste component of pomegranate juice production, has been
40 reported to contain 249.4 mg/g phenolic compounds (tannic acid equivalents), while the arils
41 contain 24.4 mg/g (Li et al., 2006). It is particularly rich of hydrolysable tannins, specifically
42 punicalagins, which are multiple esters of gallic acid and glucose known for their antioxidant,
43 antimicrobial and anti-inflammatory properties (Foss et al., 2014; Jurenka, 2008; Kharchoufi et al.,
44 2018; Pagliarulo et al., 2016).

45 Pomegranate is symbolic of fruits that are rich in phenolic compounds which have gained increased
46 popularity over the past three decades. Due to pomegranate's reported health properties, the global
47 demand for its processed products has spiked in recent years triggering the expansion of
48 pomegranate processing industry worldwide (Hasnaoui, Wathelet, & Jiménez-Araujo, 2014). For
49 the most part, the fruit is today industrially used for juice extraction, a process that generates large
50 amounts of waste, mainly PgP: 50% of the three million tons of pomegranate processed annually
51 worldwide (Qu W. et al., 2009; Teixeira da Silva et al., 2013). Due to its high organic matter
52 content, PgP is subjected to the EU Directive 2008/98/EC (2008), which prohibits land disposal and
53 landfilling, as this poses an environmental risk of uncontrolled greenhouse gas production as well
54 as a threat to watercourses (Qu W. et al., 2009). Food industries have therefore to rethink their
55 waste management systems, mainly based on massive landfilling, and turn more sustainable
56 strategies aimed at reducing the amount of waste to be disposed. A great way of achieving this goal
57 is by turning fruit and vegetable wastes into added-value resources through the recovery and use of

58 the beneficial components they contain in agricultural, food, cosmetic and/or pharmaceutical
59 industries. It is also a circular economy approach in waste management, one that supports
60 sustainable food production and consumption and that participates in reducing the socio-economic
61 and environmental impacts of food wastes (Sagar, Pareek, Sharma, Yahia, & Lobo, 2018; Vilariño,
62 Franco, & Quarrington, 2017).

63 While the antioxidant activity of pomegranate has been the subject of numerous investigations,
64 little is known regarding its antimicrobial activity. It has been reported however that PgP extracts
65 have higher antimicrobial activity than extracts from juice, seeds or (Dahham, Ali, Tabassum, &
66 Khan, 2010), and that the antimicrobial spectra obtained are typically broad including Gram-
67 positive and Gram-negative bacteria as well as yeasts (Bharani & Namasivayam, 2016; Ferrazzano
68 et al., 2017; Pagliarulo et al., 2016). The growing concern among consumers and food processors
69 for the possible health hazards of synthetic food additives has led to the search for more natural
70 antimicrobials as alternatives to chemical additives such as butylated hydroxyanisole, butylated
71 hydroxytoluene and propyl gallate which have been linked to health issues (Echegaray et al., 2018;
72 Estévez & Luna, 2017). A similar search for new antimicrobials is also going on in the
73 pharmaceutical sector sparked by the alarming increase in antimicrobial resistance among
74 pathogens (Fair & Tor, 2014). Being able to use PgP as a source of antimicrobial compounds that
75 can be applied, not only to food products, but also to cosmetic and pharmaceutical formulations is
76 therefore an attractive prospect.

77 Yet, the compounds behind the antimicrobial activity of pomegranate have yet to be clearly
78 identified. To date, no two studies have confirmed the presence of the same antimicrobial
79 compound in pomegranate, despite the fact that the identified compounds are among the most
80 commonly found in pomegranate (Read, Deseo, Hawes, & Rochfort, 2019). Discrepancies may be
81 due to differences in the methods used, but they may also be linked to different phytochemical
82 compositions between plants of different varieties and/or geographical regions (Mekni et al., 2018).

83 It appears therefore that there is a need for an analytical investigation in order to clarify this issue.
84 The present study sets out to investigate and identify the antimicrobial molecules present in PgP

85 extracts of two varieties of pomegranate (Zheri and Hicaznar) originating from two geographical
86 regions (Morocco and Turkey).

87

88 **2. Materials and methods**

89 *2.1. Chemicals and media*

90 All solvents used in this study were high purity solvents. Methanol was purchased from Honeywell
91 (Levallois Perret, France), acetonitrile and formic acid were purchased from Sigma-Aldrich (Lyon,
92 France), acetone and heptane were purchased from Carlo Ebra (Val-de-Reuil, France) and ethanol
93 was purchased from Thermo Fisher Scientific (Illkirch-Graffenstaden, France). Ultra-pure water
94 was produced by a Milli-Q system (Synergy UV; Millipore SAS, Molsheim, France). Punicalagin
95 standard (mixture of α and β anomers) was acquired from Sigma-Aldrich. Mueller-Hinton agar
96 medium was purchased from VWR International (Radnor, PA, USA), de Man Rogosa and Sharpe
97 (MRS) broth was purchased from Biokar Diagnostics (Beauvais, France) and Brain Heart Infusion
98 agar was purchased from Bio-Rad (Marnes-La-Coquette, France).

99

100 *2.2. Microbial strains*

101 The microbial strains used in the present study are listed in Table 3. All cultures were maintained as
102 frozen stocks held at -80°C in Cryobank cryogenic beads (Bio-Rad, Hercules, CA, USA). For short
103 term storage, strains were subcultured on brain heart infusion agar and kept at 4°C . Before
104 experimental use, the strains were cultivated twice at 37°C for 18 to 24 h in MRS broth. Culturing
105 was done without aeration.

106

107 *2.3. Samples*

108 Peels of fresh *Punica granatum* L. ‘Zhéri’ (a Moroccan variety with a sweet taste) and *Punica*
109 *granatum* L. ‘Hicaznar’ (a Turkish variety with a sour-sweet taste) were manually separated from
110 the rest of the fruit, freeze-dried, crushed into a fine powder using a freezer mill and stored at –
111 80°C for further use.

112

113 *2.4. Solid-liquid extraction*

114 To determine the solvent that would extract high levels of antimicrobial compounds, solid-liquid
115 extraction was performed using solvents over a wide polarity range. Tested solvents consisted of
116 water, water/ethanol (1:1; v/v), ethanol, acetone and heptane. Two grams of sample were mixed
117 with 10 ml of solvent, sonicated at ambient temperature for 20 minutes and centrifuged at 5000 rpm
118 for 10 min. The supernatant was then filtered through a 0.45 µm pore-size polyester membrane.

119

120 *2.5. Solid phase extraction*

121 Solid phase extraction was used as a fractioning step. First, pomegranate peel extracts were diluted
122 10 fold in ultra-pure water. Then extraction was carried out on a 1-g SPE cartridge made of a
123 divinylbenzene and vinyl pyrrolidone co-polymer (Oasis HLB; Waters, Dublin, Ireland). The
124 sorbent was conditioned with 10 ml of methanol and equilibrated with 10 ml of 10% methanol in
125 ultra-pure water. Fifty ml of the diluted extract were loaded on the cartridge, and the sorbent was
126 washed with 10 ml of 10% methanol in ultra-pure water. Successive elutions were then performed
127 with 10 ml of 20 %, 30 %, 40 %, 50 %, 60 %, 70 %, 80 %, 90 % and 100 % methanol in ultra-pure
128 water. All the fractions were collected separately and tested for antimicrobial activity (see Section
129 2.7). Active fractions were pooled and used for composition determination and purification of
130 antimicrobial compounds.

131

132 *2.6. Bioassay-guided purification*

133 *Step 1.* Pooled active fractions from SPE were subjected to reverse-phase HPLC using a 2695 series
134 Alliance HPLC system (Waters) equipped with a C18 PFP column (250 mm x 4.6 mm i.d., 5 µm;
135 ACE, Aberdeen, Scotland) and controlled with the Empower software (Waters). The sample
136 injection volume was set at 100 µl. A binary mobile phase gradient with 0.1% formic acid in ultra-
137 pure water (A) and 0.1% formic acid in acetonitrile (B) was used at a flow rate of 1 ml.min⁻¹. The
138 following elution gradient was used: 0 to 5 min, 0 to 10% B; 5 to 15 min, 10 to 15% B; and 15 to

139 50 min, 15 to 90% B. The detection was achieved on a Photodiode Array Detector by scanning
140 wavelengths from 200 to 400 nm and 3D UV spectra were recorded. Fractions (0.5 ml or 30 s) were
141 collected with a Roucaire Cygnet collector and concentrated by vacuum evaporation using a
142 SPD12P SpeedVac Savant concentrator (Thermo Fisher Scientific; Waltham, MA, USA) before
143 being tested for antimicrobial activity (see Section 2.7).

144 *Step 2.* Active fractions were further purified by UHPLC on a UPLC Acquity system (Waters)
145 equipped with an Acquity Phenyl BEH column (100 mm x 2.1 mm i.d., 1.7 μm ; Waters) and
146 controlled by the Compass 1.3 software for micrOTOF (Bruker; Billerica, MA, USA). The sample
147 injection volume was set at 10 μl . The following mobile phase gradient was used at a flow rate of 1
148 $\text{ml}\cdot\text{min}^{-1}$: 0 to 15 min, 2 to 20 %B ; 15 to 18 min, 20 to 30 %B; and 18 to 20 min, 30 to 95 %B.
149 Detection and fraction (0.06 ml or 12 s) collection were achieved as described in Step 1.

150

151 *2.7. Antimicrobial activity assay*

152 The determination of antimicrobial activity was done by the agar diffusion assay as described by
153 Izquierdo et al. (2008) with slight modifications. This test is based on the diffusion of antimicrobial
154 agents in an agar culture medium resulting in clear zones of inhibition of the cultured
155 microorganism growth. Five ml of Mueller-Hinton agar melted and inoculated with 50 μl of a
156 bacterial strain (indicator strain) were poured in a Petri dish. After solidification and drying for 15
157 min, 10 μl of the pomegranate peel extracts or purified compounds were dropped on the agar.
158 Extraction solvents were tested as blanks. After an overnight incubation at 37°C, Petri dishes are
159 examined for the presence of inhibition zones. For a quick assessment of the antimicrobial activity,
160 the diameter of the observed clear zones was measured. Alternatively, titration of the activity was
161 performed by testing the activity of 2-fold serial dilutions of an initial solution. A minimal
162 inhibitory concentration (MIC) was then determined as the smallest concentration showing activity
163 against the indicator strain. Strains with the highest sensitivity to pomegranate extracts were
164 selected as indicator strains.

165

166 2.8. *Mass spectrometry*

167 Active fractions from SPE were analyzed by mass spectrometry (MS) using a micrOTOF-QII
168 (Bruker, Billerica, MA, USA) mass spectrometer consisting of an electrospray ionization source
169 (ESI), a quadrupole (Q) and a time of flight analyzer, and hyphenated with a UHPLC system. ESI
170 was operated in positive mode and the spectra were acquired in “full scan” mode in the range of
171 m/z 100 - 2000. The electrospray conditions were: nebulizer pressure of 40.2 psi; drying gas flow
172 of 8.5 L/min at 200 °C; capillary voltage of 4000 V. The chromatographic system and conditions
173 were as described in section 3.5, Step 2.

174

175 **3. Results and discussion**

176 *3.1. Extraction solvent and target bacterial strain*

177 Solvents with different polarity indices were tested for solid-liquid extraction of antimicrobials
178 from PgP and the obtained extracts were tested using the agar diffusion assay against strains of
179 *Pseudomonas aeruginosa* ATCC 9027 and *Staphylococcus epidermidis* ATCC 12228. The
180 diameters of the obtained clear zones were indicative of the extent of the antimicrobial activity in
181 the extracts. An illustration is shown in Figure 1 with *S. epidermidis* ATCC 12228 as a target. The
182 results showed that with both pomegranate varieties, Zhéri and Hicaznar, the highest antimicrobial
183 activity was obtained in ethanol extracts with diameters of inhibition zones up to 20 mm, followed
184 by a water/ethanol mixture then water (Table 1). This indicated that the antimicrobial compounds in
185 PgP are of a polar nature, which allows their extraction using a simple, green and economic method
186 based on ethanol and/or water. This is highly important when it comes to large-scale human or
187 industrial applications.

188 It is worth noting that the used solvents did not show any activity against the used target strains in
189 the agar diffusion assay conditions (Table 1). The observed activity was therefore due solely to the
190 active compounds contained in PgP. Based on these results, ethanol was chosen as the extraction
191 solvent in this study.

192 As far as the target bacteria are concerned, activity was observed against *P. aeruginosa* ATCC
193 9027 (a Gram-negative species) and *S. epidermidis* ATCC 12228 (a Gram-positive species) which
194 were both sensitive to the three active extracts. *S. epidermidis* ATCC 12228 was chosen as the
195 target strain for further experiments as it gave clear and easily measurable inhibition zones. *P.*
196 *aeruginosa* ATCC 9027 on the other hand gave zones with a blurred limit, which is generally
197 linked to the growth of bacterial cells developing some level of resistance near the edge where the
198 antimicrobial's concentrations are low.

199

200 3.2. Composition of the extracts

201 Ethanol extracts of sweet and sour-sweet PgPs were subjected to co-polymer-based SPE with a
202 multi-step elution consisting of increasing amounts of methanol in water. Only the fractions
203 collected between 40 and 80% of methanol showed antimicrobial activity. They were pooled and
204 analyzed by UHPLC-ESI-MS-TOF for composition determination. The chromatograms obtained
205 with extracts from sweet and sour-sweet PgPs are given Figure 2. Compounds were identified
206 based on UV and MS spectra, relative retention times and data collected from the literature. The
207 identified compounds in both extracts are listed in Table 2. The results showed that PgP extracts
208 were mainly made of phenolic compounds such as pedunculagins I and II, ellagic acid, ellagic acid-
209 O-pentoside, and punicalagins α and β , which is in line with previous findings (Fischer, Carle, &
210 Kammerer, 2011; Hernández-Corroto, Marina, & García, 2019; Kharchoufi et al., 2018; Lu, Ding,
211 & Yuan, 2008; Pagliarulo et al., 2016).

212 Among the identified compounds in the ethanolic extract of sweet PgP, the major components are
213 the hydrolysable tannins punicalagin α and punicalagin β . Identification of punicalagin was based
214 on its MS spectrum which showed an exact monoisotopic mass is 1084.066 amu, and a 1067.0625
215 m/z peak corresponding to its molecular ion $[M+H-H_2O]^+$ (mass precision of 1.3 ppm) (Figure 3B).
216 In-source-fragmentation showed a 765.0545 m/z peak corresponding to the punicalin $[M+H-H_2O]^+$
217 ion and a 303.0158 m/z peak corresponding to the ellagic acid $[M+H]^+$ ion.

218 Our results also showed that the overall compositions of the ethanolic extracts from PgPs of the
219 sweet and sour-sweet varieties were very similar despite taxonomic and geographic differences.
220 Only the relative proportions of the identified compounds did vary between the two samples.
221 Similar data has been obtained regarding the phenolic content of pomegranate juices of different
222 varieties (Labbé et al., 2016; Mekni et al., 2018).

223

224 3.3. Activity-guided purification and identification of the antimicrobial compounds

225 Reverse-phase HPLC separation of sweet and sour-sweet PgPs extracts followed by fraction
226 collection and activity testing yielded very similar chromatograms with two fractions independently
227 active against *S. epidermidis* ATCC 1222. Figure 3A shows an example with the sweet PgP extract.
228 Mass analysis of the two fractions showed the same mass spectrum corresponding to punicalagin
229 (Figure 3B). The two fractions corresponded in fact to α and β anomers of punicalagin. Although
230 higher peak intensities were obtained with the sour-sweet Hicaznar variety than with the sweet
231 Zhéri variety, no conclusion could be drawn as to a difference in punicalagin content between the
232 two varieties since extraction and analysis steps in this study were not quantitative. Following
233 HPLC separation of PgP extracts, active fractions were pooled and further separated by UHPLC.
234 As with HPLC separation, only two independently active fractions, corresponding to
235 punicalagin's α and β anomers, were obtained.

236 When each of the two active fractions, containing either anomer of punicalagin, was separately
237 analyzed by UHPLC, we observed the systematic presence of both anomers. This is due to the α/β
238 inter-conversion of the glucose in the punicalagin molecule (Kraszni, Marosi, & Larive, 2013). This
239 mutarotation reaction is possible when glucose is linearized prior to a new hemiacetalisation which
240 closes the sugar in one anomeric form or the other (Silva, da Silva, & da Silva, 2006). In our
241 separation conditions, an α/β equilibrium is reached at a 3/7 ratio, which is close to the 1/2 ratio
242 reported by Barbieri & Heard (2019) in a H₂O/MeOH mixture.

243 Punicalagin was the only extracted compound from sweet and sour-sweet PgP with antibacterial
244 activity against the target strain *S. epidermidis* ATCC 12228. This finding is supported by reports

245 on the antimicrobial activity of hydrolysable tannins, especially punicalagin (Ekambaram, Perumal,
246 & Balakrishnan, 2016; Xu et al., 2017) which is found not only in pomegranate but also in other
247 plants such as in the leaves of *Lafoensia pacari*, a Brazilian medicinal plant (Carneiro et al., 2016),
248 and of Indian almond tree *Terminalia catappa* (Lin, Hsu, Lin, Hsu, & Hsu, 1998). Besides its
249 antimicrobial activity, punicalagin has also been reported to possess antioxidant (Seeram et al.,
250 2005), antiproliferative (Seeram et al., 2005), angiogenic (Carneiro et al., 2016), and
251 hepatoprotective (Lin et al., 1998) properties.

252 As opposed to the data presented here, a recent investigation by Khan et al. (2017) has identified
253 valoneic acid dilactone, monogalloyl-hexoside, hexahydroxydiphenoyl-hexoside and coumaric acid
254 as the antibacterial compounds in PgP, but surprisingly not punicalagin (Khan et al., 2017). In our
255 case, no activity was observed in the fractions containing valoneic acid dilactone and monogalloyl-
256 hexoside, while hexahydroxydiphenoyl-hexoside and coumaric acid were not identified in the
257 extracts studied. It is noteworthy however that in the study of Khan et al. (2017), active peel
258 extracts have been fractionated and analyzed by MS/MS to identify the compounds they contain,
259 but antimicrobial tests have not been done on the individual compounds to confirm their activity. In
260 this study, no observation was made that could suggest that antimicrobials other than punicalagin
261 may be present, such as for instance a difference in the activity spectrum between crude extracts
262 and pure fractions. Yet, antimicrobial compounds other than punicalagin may be present in PgP but
263 in small amounts, with low activity and/or that target microbial strains other than those tested in
264 this study. The presence of such compounds, which may go undetected, should therefore not be
265 ruled out.

266

267 3.4. Antimicrobial spectrum of punicalagin

268 Pure punicalagin in its two anomeric forms was tested against a range of target microbial strains
269 mainly representative of common foodborne pathogens, as well as clinically related enterococcal
270 species and a yeast species. Antimicrobial activity was observed against two out of three Gram-
271 negative and 10 out of 13 Gram-positive bacteria (Table 3). MIC values of punicalagin varied

272 between 0.3 and 1.2 $\mu\text{g}\cdot\text{ml}^{-1}$, with no apparent differences in sensitivity according to the Gram type.
273 Activity was also observed against a yeast strain of *Candida albicans*. No activity however of
274 punicalagin was observed up to 2.4 $\mu\text{g}\cdot\text{ml}^{-1}$ against tested strains of *Bacillus cereus*, *Lactobacillus*
275 *plantarum*, *Enterococcus faecium* and *Salmonella enteritidis*. This could be due to a strain-specific
276 action of punicalagin which may be more or less efficient against strains of the same species.
277 Previous studies have in fact shown activity of pomegranate peel extracts against strains of species
278 which showed no sensitivity in the present study (Al-Hassnawi, 2017; Al-Zoreky, 2009). Yet, the
279 observed spectrum of activity of punicalagin is broad one targeting both Gram-positive and Gram-
280 negative bacteria, as well as a yeast strain.

281

282 **4. Conclusions**

283 The use of fruit and vegetable wastes as a renewable bioresource for the production of high-value
284 bioactive compounds whilst also addressing the huge problem of food waste disposal is an
285 appealing prospect. This contributes towards a circular economy way of managing human wastes
286 and is in line with the goals of sustainable development. The present study shows that punicalagin
287 contained in pomegranate peels is at the origin of their antimicrobial activity. These waste peels
288 could therefore be a source of an antimicrobial compound with potential applications in the food
289 and pharmaceutical industries.

290

291 **Declaration of Competing Interest**

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

294

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298

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431

432

433 **Figure legends**

434

435 **Figure 1.** Agar diffusion antimicrobial assay against *S. epidermidis* ATCC 12228 with extracts
436 from sweet (A) and sour-sweet (B) PgP.

437 **Figure 2.** UHPLC-ESI-MS-TOF chromatograms in positive mode obtained with active fractions
438 from SPE of ethanolic extracts from sweet (A) and sour-sweet (B) PgP.

439 **Figure 3.** HPLC-DAD ($\lambda_{\text{DAD}} = 260 \text{ nm}$) chromatogram of pooled active fractions from SPE of
440 sweet PgP ethanolic extract (A). MS analysis of the collected active fractions (highlighted) showed
441 that each contained punicalagin but under a different anomeric form. Only the MS spectrum and
442 molecular identity for the first peak (α anomer) are shown here (B).

443

444 **Table 1**445 Diameters (mm) of inhibition zones obtained with sour-sweet and sweet PgP extracts tested on *P.*446 *aeruginosa* ATCC 9027 and *S. epidermidis* ATCC 12228

447

Extraction solvent	<i>P. aeruginosa</i> ATCC 9027			<i>S. epidermidis</i> ATCC 12228		
	Blank	Sweet PgP	Sour-sweet PgP	Blank	Sweet PgP	Sour-sweet PgP
Water	0	14	14	0	13	16
Water/ethanol (1:1; v/v)	0	16	17	0	14	17
Ethanol	0	20	19	0	17	17
Acetone	0	0	0	0	0	0
Heptane	0	0	0	0	0	0

448

449

450

451 **Table 2**

452 Identified compounds in the PgP hydroethanolic extracts. Indicated retention times correspond to
 453 the chromatogram of the sweet PgP hydroethanolic extract shown in Figure 2A.

454

Compound	Retention time (min)	λ_{\max} (nm)	m/z values			References*
			[M+H] ⁺	[M+H-H ₂ O] ⁺	Other fragments	
Kaempferol-O-rutinoside	4.58	270	595	----	----	b
Punicalin derivative	4.88	260 ; 380	----	----	917 ; 765 ; 303	c, b, d
Valoneic acid-dilactone	5.35	255 ; 370	471	----	----	c, d
Peduncalagin I	5.43	260 ; 380	----	767	----	a, b, c, d, g
Punicalagin derivative	5.77	255 ; 375	----	----	1123 ; 1067 ; 303	a, b, c, d, e, f, g
Punicalagin α	6.37	260 ; 380	----	1067	765 ; 534 ; 303	a, b, c, d, e, f, g
Digalloyl-gallagyl-hexoside derivative	6.65	260 ; 375	----	----	1249 ; 1087 ; 767 ; 303	d
Peduncalagin III	7.42	260 ; 370	935	----	505 ; 303	d
Digalloyl-gallagyl-hexoside	7.89	260 ; 375	1087	----	959 ; 767 ; 303	d
Punicalagin β	8.18	260 ; 380	----	1067	765 ; 303	a, b, c, d, e, f, g
Peduncalagin II	8.43 ; 9.72	270	787	----	----	a, b, c, d, e, g
Ellagic acid derivative	8.56	270	----	----	801 ; 303	a, b, c, d, e, g
Ellagic acid-O-hexoside	9.52	255 ; 360	465	----	303	c, d, e, g
Ellagic acid-O-pentoside	11.81	255 ; 360	435	----	303	a, c, d, e, g
Ellagic acid	11.98	255 ; 370	303	----	----	a, b, c, d, e, g
Granatin B	12.36	270 ; 365	953	----	633	a, b, c
Galloyl-hexahydroxy-diphenoyl-hexoside	13.00	265	----	617	303	a, b, d
Eschweilenol C	14.55	265 ; 350	449	----	287	b

455 * a (Abid et al., 2017); b (Al-Rawahi, 2014); c (Fischer et al., 2011); d (Hernández-Corroto et al., 2019); e (Kharchoufi et al., 2018);

456 f (Lu et al., 2008); g (Pagliarulo et al., 2016).

457

458

459 **Table 3**460 Minimal inhibitory concentrations of punicalagin (α and β anomers) against various strains of

461 yeast, Gram-positive bacteria and Gram-negative bacteria.

462

Target	Strains*	MIC ($\mu\text{g}\cdot\text{ml}^{-1}$)
Yeast	<i>Candida albicans</i> ATCC 10231	1.2
Gram-negative bacteria	<i>Pseudomonas aeruginosa</i> ATCC 9027	0.60
	<i>Salmonella enteritidis</i> LC 216	> 2.4
	<i>Escherichia coli</i> ATCC 25922	1.2
Gram positive bacteria	<i>Staphylococcus epidermidis</i> ATCC 12228	0.60
	<i>Staphylococcus xylosum</i> LC 57	0.60
	<i>Staphylococcus aureus</i> ATCC 6538	0.60
	<i>Bacillus cereus</i> CIP 78.3	> 2.4
	<i>Lactobacillus sakei ssp. sakei</i> ATCC 15521	0.60
	<i>Lactobacillus plantarum</i> CECT 4185	> 2.4
	<i>Pediococcus acidilactici</i> LC P1	0.60
	<i>Enterococcus faecium</i> DSMZ 10663	> 2.4
	<i>Enterococcus faecalis</i> ATCC 19433	0.60
	<i>Enterococcus mundtii</i> LC E23	0.30
	<i>Enterococcus sulfureus</i> LC E28	0.60
	<i>Enterococcus casseliflavus</i> LC E1	0.30
	<i>Enterococcus columbae</i> LC E2	0.60

463

* LC, laboratory collection; CIP, Collection de l'Institut Pasteur; ATCC, American Type Culture Collection; CECT, Coleccion Española de Cultivos

464

Tipo ; DSMZ, Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH

465

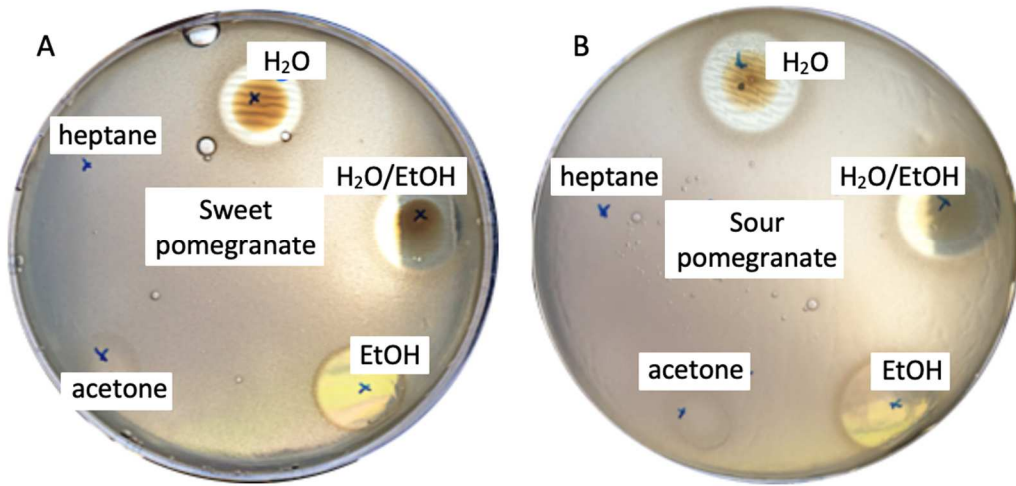


Figure 1

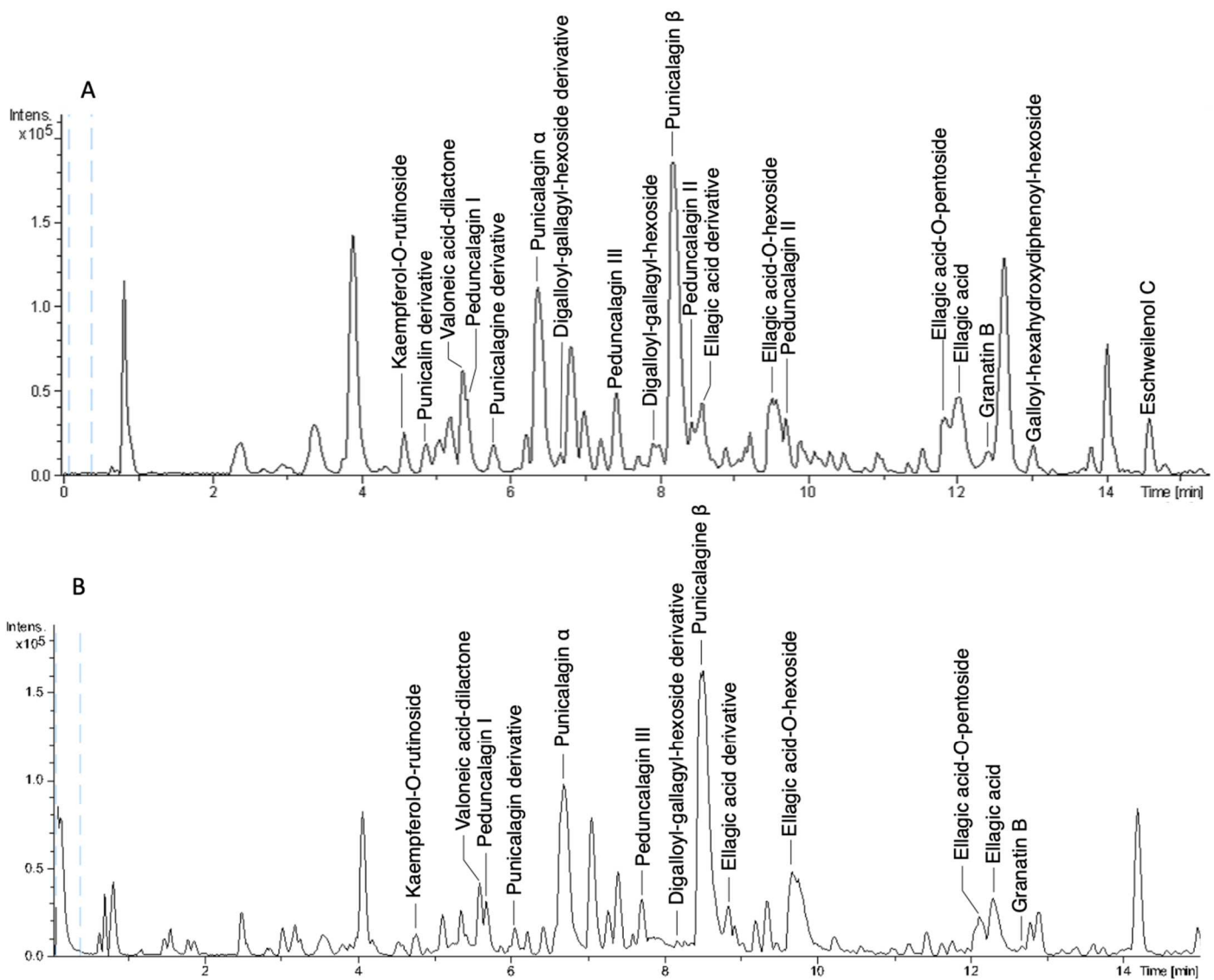
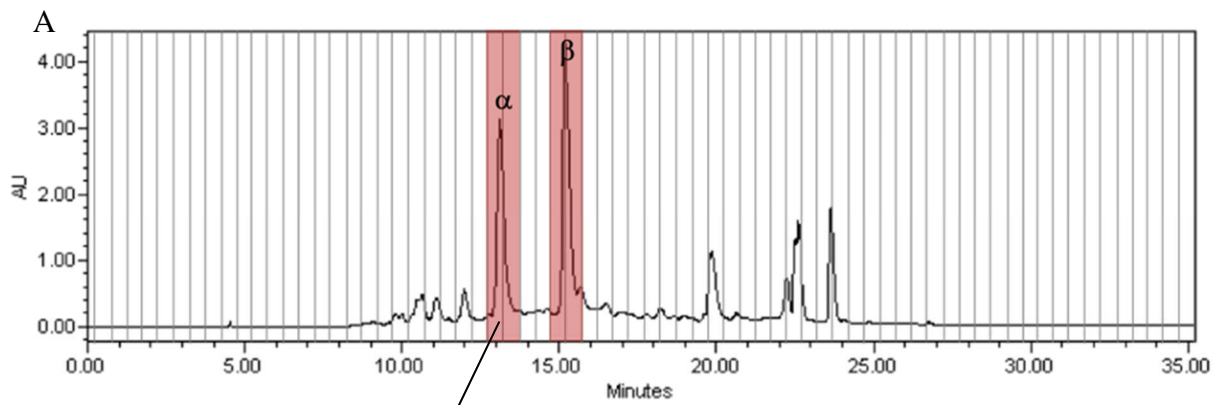


Figure 2



B

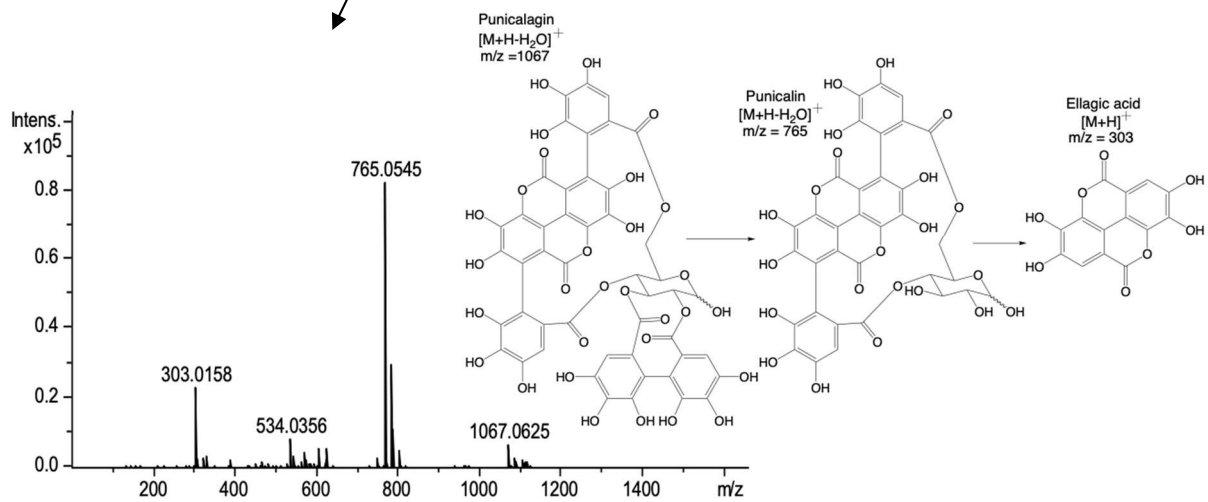


Figure 3



Fresh peels of *Punica granatum* L.

