**Supplementary Material**

# Chemical, antioxidant, and antimicrobial properties of the peel and male flowers by-products of four varieties of *Punica granatum* L. cultivated in the Marche region given their exploitation in cosmetic products

**Summary of Tables**



**Polyphenols identification**

The first large group of hydrosysable tannins identified in our work contains three main subclasses of compound: gallotannins and ellagitannins belonging to the phenol groups that are esterified to the hydroxyl groups of glucose: gallic acid in gallotannins and hexahydroxydiphenic acid (HHDP) in ellagitannins [1] and gallagyl esters.

Compound **C1** and **C2** belonging to the classes of gallotannins showing a $[M-H]^-$ ions at m/z 331 and 483, respectively. **C1** reports a fragment ions in $MS^2$ at m/z 169 related to the loss of hexose fraction (162Da) and a fragment at m/z 125 which is typical for the fragmentation of gallic acid[M-H-162 Da-44 Da]. According to the literature **C1** was assigned to galloyl-hexoside, while **C2** was identified as digalloyl-hexoside according to its precursor ion at m/z 483 [2, 3].

Among hydrolysable tannins, ellagitannins compounds from **C3** to **C18** were identified. For this class of compound, characteristic fragments ions were identified as m/z 301 and attributed to the aglycone of ellagic acid that was obtained after a spontaneous-lactonization of the HHDP residue (**C16**) [3]. So, **C16** was identified as ellagic acid showing a typical signal at m/z 301and characteristic fragment ions at m/z 229 (corresponding to the loss of four $H_2O$ molecules) and m/z 185 (corresponding to a loss of $CO_2$).

**C3** at 2.3 min. produced a molecular ion at m/z 481 $[M-H]^-$ and was identified as HHDP-hex [3-5]. The ellagic acid hexoside moiety was also detected in the compounds C4, C11, C12, C13. **C4** showed an $[M-H]^-$ ion at m/z 633 and fragments at m/z 301 and 169 which are typical for the loss of ellagic acid and the galloyl moiety. **C11** showed an $[M-H]^-$ ion at m/z 935 and a typical fragments at m/z 633 and 301 and was identified as galloyl-bis-HHDPhexoside (casuaricitin) [3]. **C12** showed an [M-H]- ion at m/z 463 and typical fragments at m/z 301 indicated the loss of hexoside and was identified as ellagic acid hex[3, 4]. **C13** exhibited an $[M\_H]^-$ ion at m/z 951 producing fragments at m/z 933 and at m/z 301 (ellagic acid) in the MS2 experiment. This fragment (m/z 933), generating fragments at m/z 915 from the loss of water, is typical for castalagin/vescalagin or galloyl-gallagyl-hexoside (galloylpunicalin, pedunculagin III) as already reported in litterature [3, 5]. **C9** produced an $[M-H]^-$ ion at m/z 799 and fragments at m/z 479 (loss of ellagic acid), fragments at m/z 301(ellagic acid). Furthermore, the established fragments of ellagic acid were confirmed. This compound may be attributed to granatin A (HHDP-DHHDP-hexoside). **C6** (m/z1415) and C15 (m/z 433) produced fragments at m/z 933 and at m/z 301 (ellagic acid) in the MS2 experiment, respectively. C6 was identified as di(HHDP-galloylglucose)-pentose[6]. **C15** was assigned to an ellagic acid-pentoside that

in the MS2 experiment, the ion at m/z 301 was generated by the loss of 132 Da, reasonably assigned as the elimination of pentose. In addition, ellagitannins with a gluconic acid core were also found in pomegranates. Among these, **C5** was identified as galloyl-HHDP-gluconic acid. Its [M−H]− ion at m/z 649 showed fragments at m/z 497 and 301, resulting from the loss of gallic acid (releasing HHDP-gluconic acid) and ellagic acid, respectively. Galloyl-HHDP-gluconic acid (m/z 649) also formed part of **C10** exhibiting an [M−H]− ion at m/z 801. Further fragments at m/z 348 and 497 resulted from the loss of ellagic acid and gallic acid. This compound was identified as digalloyl-HHDP-gluconic acid (punigluconin). Compound **C7** exhibited an [M−H]− ion at m/z 783. The loss of ellagic acid in the MS2 experiment produced fragments at m/z 481 and the fragment atm/z 301 of ellagic acid. Based on this fragmentation pathway compound **C7** was identified as bis-HHDP-hexoside (pedunculagin I). **C8** was characterised as digalloyl-HHDP-hexoside (pedunculagin II). This assignment is based on its [M−H]− ion at m/z 785 and the release of typical ellagitannin and gallotannin fragments at m/z 301 (ellagic acid) and 169 (galloyl group). Each of the two different retention times corresponded to an isomeric structure, also differing in their fragmentation patterns. The [M-H]− ion of **C15** was obtained at m/z 433. In the MS2 experiment, the ion at m/z 301 was generated by the loss of 132 Da, reasonably assigned as the elimination of pentose. The occurrence of the ion at m/z 300 was attributed to a homolytic rupture of the glycosidic bond [5]. In addition to ellagic acid pentoside, further monoglycosylated ellagic acid derivatives were observed as an ellagic acid deoxyhexoside (m/z 447; **C14**) and an ellagic acid derivate (der) (m/z 441; **C17**) all of them showing the typical fragments of ellagic acid (m/z 301). An [M−H]− ion at m/z 469 was observed for the isomeric compound **C18**, producing fragment ions at m/z 425 indicating the loss of a carboxyl group and typical fragments of ellagic acid. Compound **C18** was therefore assigned to the valoneic acid bilactone [3]. **C19** eluted at 4.0 min with a precursor ion at m/z 781 and a fragment at m/z 601 (gallagyl residue) were identified as gallagyl-hexoside (punicalin). Identification of punicalagin was based on its MS spectrum which showed an exact $[M-H]^-$ ion at m/z 1083. Punicalagin $\alpha$ and $\beta$ eluted in peaks **C20** (9.2 min) and **21** (11.7 min). MS spectra of both isomers showed a $[M-H]^-$ ion at m/z 1083, which fragmented at m/z 781 and m/z 601 (corresponding to the loss of ellagic acid and gallagic acid moiety, respectively). The 781 m/z peak **C 19** corresponded to the punicalin [M -H]- ion and a 302 m/z peak to the ellagic acid $[M-H]^-$ ion [7]. Gallic acid (**C22**) is a signal at m/z 169 and showed a typical fragment at m/z 125 corresponding to the loss of CO2. **C23** showed a signal at m/z 355 and fragment ions at m/z 193,175,217 and 236. This compound was identified as ferulic acid hex due to its fragmentation revealed the formation of an aglycone fragment at m/z 193 (ferulic acid) and fragments at m/z 175 from the loss of water. **C24** was identified as dihydrokaempferol-hex and produced an ion at m/z 449 and a fragments at m/z 287 (loss of hexose moiety), 259 and 269( loss of water). Among the class of gallotechin compounds **25** and **26** were identified as gallocatechin and catechin-gallocatechin, respectively. C25 showed a ion at m/z 306 producing a fragments at m/z 125,137,165 and 219, while C26 (m/z593) showed fragments at m/z 441,305,423 and 137 suggesting that the component of aglycone in these compounds was either catechin (290 Da) or epicatechin [2]. Compounds **C30** ansd **C31** showed an ion at m/z 447 reporting the same fragment ions at m/z 285 in the MS2 experiment. In case of **C30** were also detect the fragments ions at m/z 255 and 227 already detected fot the astralagin[2]. The **C31** were identified as luteolin-glucoside reporting a in its MS2 experiment, the fragment ions at m/z 285 which led to the identification of the aglycone as lutelolin [2]. **C29** (rt = 24.1 min) exhibited a $[M-H]^-$ ion at m/z 593. This compound was identified as kaempferol-7-orhahmano-glucoside due to in the MS2 experiment showed the fragment ion at m/z 285 and characteristic fragments at m/z 255, 227 belonging to kaempferol [2]. **C28** exhibited $[M-H]^-$ ions at m/z 609 with fragments ions at m/z 301 and 300 matched with the loss of rhamno-glucose (146 + 162 Da) and was identified as quercetin-3-O-rhahmano-glucoside (rutin) [2]. We also revealed the presence of **C27** (m/z 343, RT=1.54 min.) never found in pomegranate peel extracts, according to the literature. The identification of this compound will be further confirmed by HRMS analysis.

**Table S1.** Identification of polyphenols in pomegranate by-product extracts by UPLC-ESI-MS/MS analysis.

| Peak number | R.T. (min.) | Precursor ion *m/z* $[M-H]^-$ | $MS^2$ ion *m/z* | Identification |
|---|---|---|---|---|
| **Hydrolysable tannins** | | | | |
| Gallotannins | | | | |
| C1 | 2.6 | 331 | 125,169 | Galloyl-hex |
| C2 | 11.2 | 483 | 169,125,439 | Digalloyl-hex |
| Ellagitannins | | | | |
| C3 | 2.3 | 481 | 301,275,257 | $HHDP^2$-hex |
| C4 | 3.6 | 633 | 301, 275, 249, 169 | Galloyl-HHDP-hex |
| C5 | 3.6 | 649 | 301, 497 | Galloyl-HHDP-glucoside |
| C6 | 6.3 | 1415 | 633, 613, 783 | Di(HHDP-galloylglucose)-pent |
| C7 | 6.8 | 783 | 301, 481, 275 | Pedunculagina I |
| C8 | 11.7 | 785 | 301, 275, 249, 169 | PedunculaginaII |
| C9 | 12.9 | 799 | 479, 301, 247 | HHDP-DHHDP-hexoside Granatin A |
| C10 | 13.7 | 801 | 649, 348 | Diggalloyl-HHDP-gluc (Punigluconin) |
| C11 | 13.8 | 935 | 633, 275, 708, 301 | Galloyl-bis-HHDP-hex (Casuarinin) |
| C12 | 16.2 | 463 | 301, 300, 271, 255 | Ellagic acid-hex |
| C13 | 19.3 | 951 | 301, 933, 273, 463 | Galloyl-HHDP-DHHDP-hex (Granatin B) |
| C14 | 20.5 | 447 | 300, 301 | Ellagic acid-deoxyhex |
| C15 | 20.7 | 433 | 300, 301 | Ellagic acid-pent |
| C16 | 21.8 | 301 | 145,185,229 | Ellagic acid |
| C17 | 22.4 | 441 | 397, 398, 301 | Ellagic acid der |
| C18 | 24.2 | 469 | 425, 301, 426 | Valoneic acid bilactone |
| **Gallagyl esters** | | | | |
| C19 | 4.0 | 781 | 601, 721 | Punicalin |
| C20 | 9.2 | 1083 | 601, 302, 781, 575 | Punicalagin |
| C21 | 11.7 | 1083 | 601, 302, 781, 575 | Punicalagin |
| **Hydroxybenzoic acids** | | | | |
| C22 | 3.8 | 169 | 125 | Gallic acid |
| **Hydroxycinnamic acids** | | | | |
| C23 | 2.7 | 355 | 193, 175, 217, 236 | Ferulic acid-hex |
| **Dihydroflavonol** | | | | |
| C24 | 16.4 | 449 | 287, 259, 269 | Dihydrokaempferol-hex |
| **Gallotechin** | | | | |
| C25 | 6.8 | 306 | 125, 137, 165, 219 | Gallocatechin |
| C26 | 7.0 | 593 | 441, 305, 423, 137 | Catechin-gallocatechin |
| C27 | 1.54 | 343 | 181, 149, 113, 119, 89,59 | Unknown |
| C28 | 3.2 | 609 | 301, 300, 271 | Quercetin rutinoside |
| C29 | 24.1 | 593 | 285,255,227 | Kaempferol rutinoside |
| C30 | 25.4 | 447 | 255,227,285 | Astralagine |
| C31 | 26.3 | 447 | 285 | Luteolin-glucoside |

hex=hexoside; HHDP=hexahydroxydiphenoyl; pent=pentoside; der=derivate

The ESI source in positive ion mode was selected to detect anthocyanins extracted from pomegranate peel and male flowers extracts. MS and MS2 spectra of A1-A4 peaks showed

molecular ions characteristic of cyanidin (m/z 287), pelargonidin (m/z 271) confirmed by external standards. The anthocyanins revealed the typical mass spectrometric behavior in ESI(+)-experiments, i.e. they showed M $^{+}$experiments and the sequential loss of their saccharide moieties, releasing the aglycones in the MS+ ions.

**Table S2.** Identification of anthocyanins in pomegranate by-products extracts by UPLC-ESI-MS/MS analysis.

| Peak number | R.T. (min.) | Precursor ion *m*/*z* [M]+ | $MS^2$ ion *m*/*z* | Identification |
|---|---|---|---|---|
| A1 | 6.2 | 611 | 287, 449 | Cyanidin 3,5-diglucoside |
| A3 | 8.4 | 595 | 433 | Pelargonidin 3,5-diglucoside |
| A2 | 10.3 | 449 | 487 | Cyanidin 3-glucoside |
| A4 | 12 | 433 | 271 | Pelargonidin 3-glucoside |

**References**


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