

## **LC-ESI-MS/MS detecting conditions and results of pomegranate extract (PE)**

### **Liquid chromatography–electrospray ionization–mass spectrometry (LC-ESI-MS/MS) conditions and apparatus:**

10  $\mu$ l sample was injected into ultra-performance liquid chromatography (UPLC) instrument equipped with reverse phase C-18 column (ACQUITY UPLC - BEH C18, 1.7  $\mu$ m particle size - 2.1  $\times$  50 mm Column). Sample mobile phase was prepared by 0.2  $\mu$ m filter membrane disc and degassed by sonication before injection. Mobile phase elution was made using gradient mobile phase comprising two eluents: eluent A is 0.1% formic acid in water and eluent B is 0.1% formic acid in methanol. Elution was performed using the following gradient: 0-2 min (90%) A: (10%) B, 2-5 min (70%) A: (30%) B, 5-10 min. (30%) A: (70%) B, 15-25 min (10%) A: (90%) B, 25-29 min (100%) B, finally, 29-32 min (90%) A: (10%) B. The flow rate of the mobile phase was 0.2 ml/min, and the injection volume was 10  $\mu$ l.

The LC-ESI-MS/MS analysis was carried out using a triple quadrupole mass spectrometer (Xevo TQ-D, Waters Corp., Milford, MA, USA) with an electrospray ionization (ESI) source. The mass spectrometer was operated in negative ion mode with the following conditions: source temperature 150  $^{\circ}$ C, cone voltage 30 eV, capillary voltage 3 kV, de-solvation temperature 440  $^{\circ}$ C, cone gas flow 50 L/h, and de-solvation gas flow 900 L/h. Mass spectra were detected in the ESI between 100–1000 m/z. The peaks and spectra were processed using the Masslynx 4.1 software and tentatively identified by comparing their retention times ( $R_t$ ), mass spectra, and MS/MS fragmentation of the peaks with those of reported data.

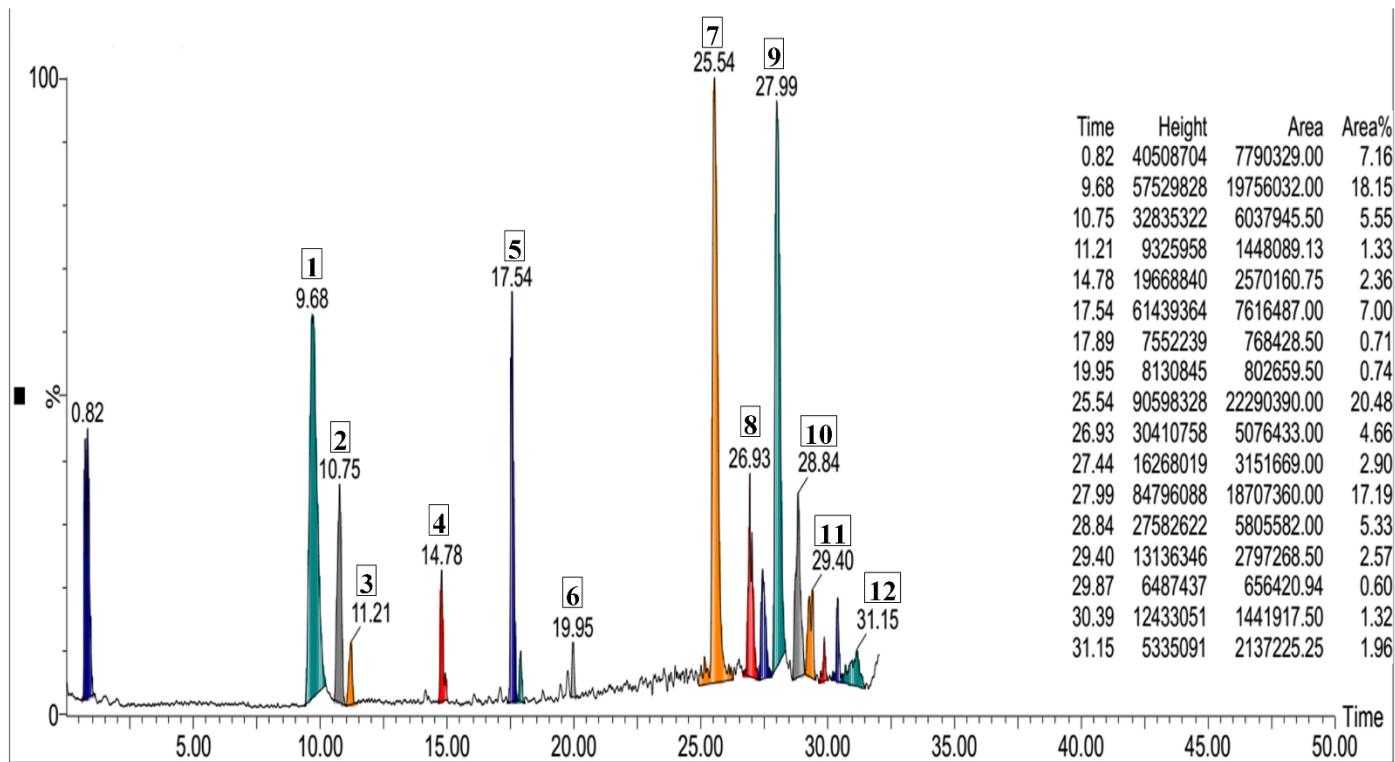
## Results

### Identification of major phytoconstituents in PE using LC-ESI-MS/MS

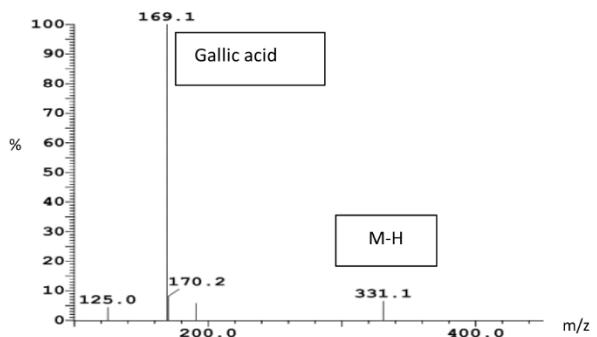
LC-ESI/MS/MS of PE revealed that twelve compounds were detected in the negative mode, of which nine were tentatively identified. The identified compounds were: Ellagic acid and its derivative (methyl ellagic acid), gallic acid, digalloyl hexoside, galloyl-hexoside, punicalin, pedunculagin, corilagin, and quercetin. Among them, ellagic acid and its derivative were the major identified compounds, accounting for 37.67 %, as depicted in **Supplementary Table 1** and **Supplementary Figure 1** and **Figure 2**.

**Supplementary Table 1. Major phytoconstituents in PE identified by LC-ESI-MS/MS.**

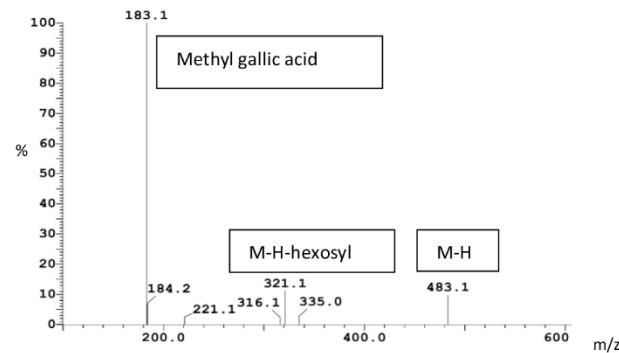
Peak number	Retention time ( $R_t$ ) (min)	M-H	Fragmentation MS <sup>2</sup>	Tentative assignment of the peaks	Chemical class	% Area	Reference
1	9.68	169	169, 125	Gallic acid	Phenolic acid	18.15	(Mena et al. 2012)
2	10.75	483	183, 184, 221, 316 321, 335, 483	Digalloyl hexoside	Phenolic acid derivative	5.55	(Gan et al. 2018)
3	11.21	519	358, 303, 195	Unidentified		1.33	
4	14.78	331	169, 125	Galloyl-hexoside	Phenolic acid derivative	2.36	(Liu and Seeram 2018)
5	17.54	781	601, 482, 331, 191	Punicalin	Ellagitannin	7.00	(Yan et al. 2017)
6	19.95	473	274 (100%), 256	Unidentified		0.74	
7	25.54	300	300	Ellagic acid	Phenolic acid	20.48	(Singh et al. 2016; Abid et al. 2017)
8	26.93	783	601, 481, 169	Pedunculagin	Ellagitannin	4.66	(Abid et al. 2017)
9	27.99	315	300, 299	Methyl Ellagic acid	Phenolic acid derivative	17.19	(Singh et al. 2016)
10	28.84	633	291 (100%)	Corilagin (Galloyl HHDP –hexoside)	Ellagitannin	5.33	(Salih et al. 2017; Radenkovs et al. 2018)
11	29.40	365	203, 133, 119	Unidentified		0.6	
12	31.15	302	301 (100%), 255, 144, 106	Quercetin	Flavonoid	1.96	(Li et al. 2016)



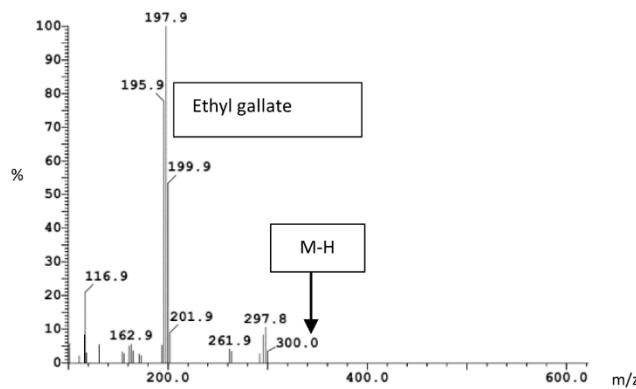
**Supplementary Figure 1. Total ion chromatogram (TIC) for PE with Area % for each compound**



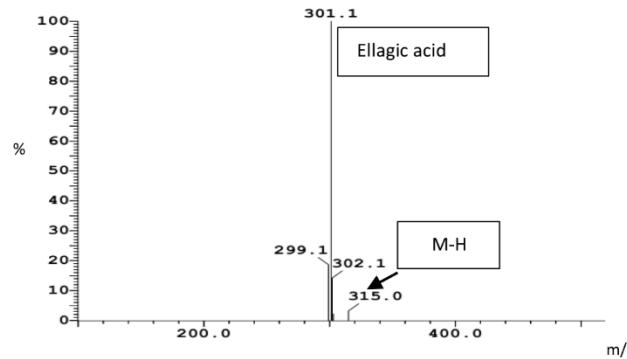
Galloyl hexoside



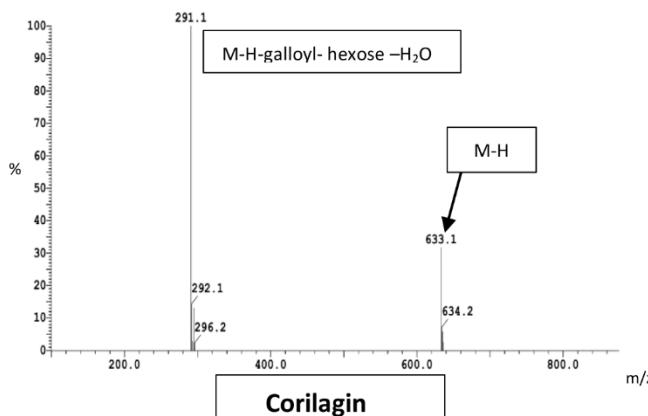
Digalloyl hexoside



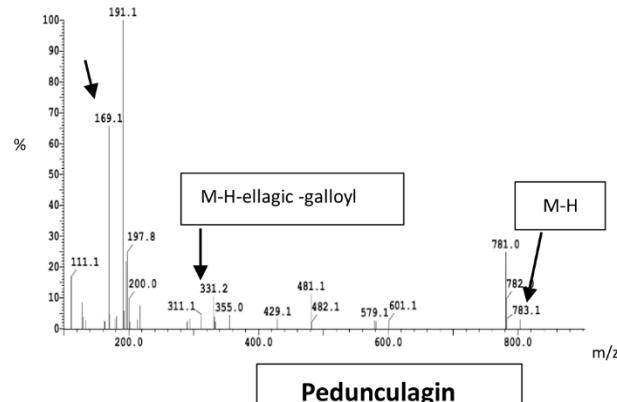
Ellagic acid



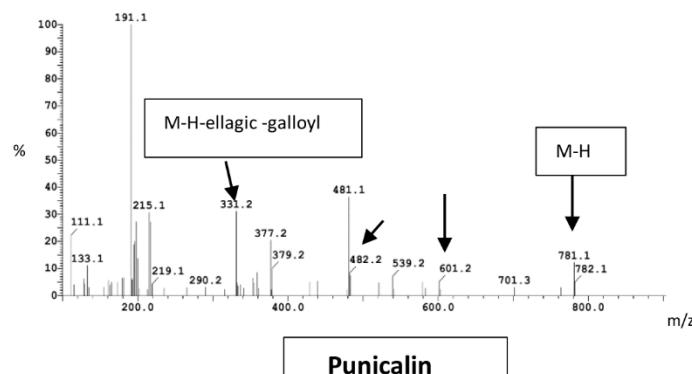
Methyl ellagic acid



Corilagin



Pedunculagin



Punicalin

Supplementary Figure 2.  $MS^2$  fragmentation for the major identified compounds in PE.

## References

Abid M, Yaich H, Cheikhrouhou S, Khemakhem, I, Bouaziz M, Attia H, Ayadi MA. 2017. Antioxidant properties and phenolic profile characterization by LC-MS/MS of selected Tunisian pomegranate peels. *J Food Sci Technol.* 54: 2890-2901.

Gan RY, Kong KW, Li HB, Wu K, Ge YY, Chan, CL, Shi XM, Corke H. 2018. Separation, identification, and bioactivities of the main gallotannins of red sword bean (*Canavalia gladiata*) coats. *Front Chem.* 6:39.

Li ZH, Guo H, Xu WB, Ge J, Li X, Alimu M, He DJ. 2016. Rapid identification of flavonoid constituents directly from PTP1B inhibitive extract of raspberry (*Rubus idaeus L.*) leaves by HPLC-ESI-QTOF-MS-MS. *J Chromatograph Sci.* 54: 805-810.

Liu Y, Seeram NP. 2018. Liquid chromatography coupled with time-of-flight tandem mass spectrometry for comprehensive phenolic characterization of pomegranate fruit and flower extracts used as ingredients in botanical dietary supplements. *J Sep Sci.* 41: 3022-3033.

Mena P, Calani L, Dall'Asta C, Galaverna G, García-Viguera C, Bruni R, Crozier A, Del Rio D. 2012. Rapid and comprehensive evaluation of (poly) phenolic compounds in pomegranate (*Punica granatum L.*) juice by UHPLC-MSn. *Molecules.* 17: 14821-14840.

Radenkova V, Püssa T, Juhnevica-Radenkova K, Anton D, Seglina D. 2018. Phytochemical characterization and antimicrobial evaluation of young leaf/shoot and press cake extracts from *Hippophae rhamnoides L.* *Food Biosci.* 24: 56-66.

Salih E, Fyhrquist P, Abdalla A, Abdelgadir A, Kanninen M, Sipi M, Luukkanen O, Fahmi M, Elamin M, Ali HA. 2017. LC-MS/MS tandem mass spectrometry for analysis of phenolic compounds and pentacyclic triterpenes in antifungal extracts of *Terminalia brownii* (Fresen). *Antibiotics (Basel).* 6: 37.

Singh A, Bajpai V, Kumar S, Sharma KR, Kumar B. 2016. Profiling of gallic and ellagic acid derivatives in different plant parts of *Terminalia arjuna* by HPLC-ESI-QTOF-MS/MS. *Nat Prod Comm.* 11: 1934578X1601100227.

Yan L, Zhou X, Shi L, Shalimu D, Ma C, Liu Y. 2017. Phenolic profiles and antioxidant activities of six Chinese pomegranate (*Punica granatum L.*) cultivars. *Int J Food Prop.* 20: S94-S107.