

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 μ l sample was injected into ultra-performance liquid chromatography (UPLC) instrument equipped with reverse phase C-18 column (ACQUITY UPLC - BEH C18, 1.7 μ m particle size - 2.1 \times 50 mm Column). Sample mobile phase was prepared by 0.2 μ 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 μ 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 Maslynx 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.

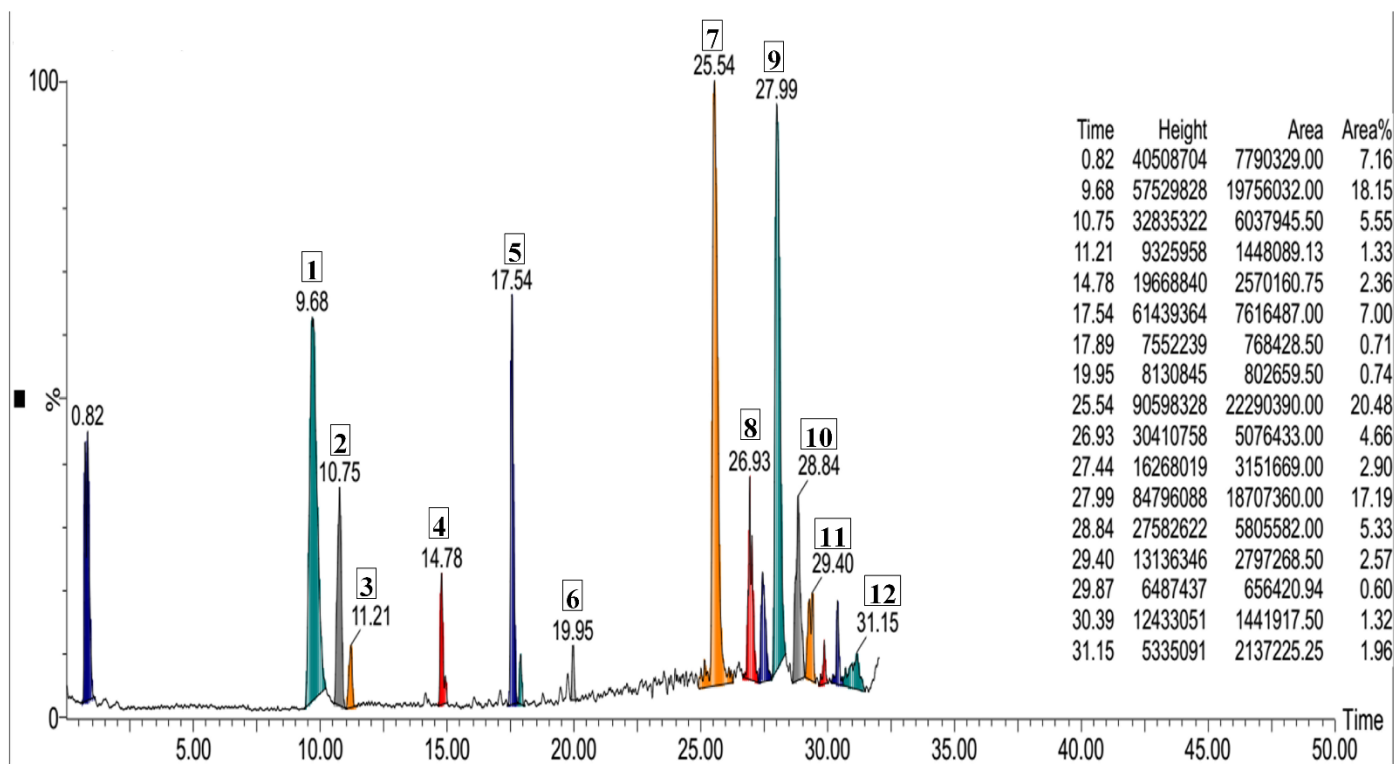
Results

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

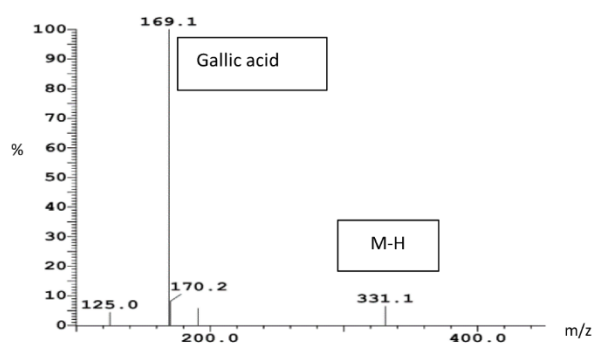
LC-ESI/MS/MS of PE revealed that twelve compounds were detected in the negative mode, of which nine were tentatively identified. The identified compounds were: Ellagic acid and its derivative (methyl ellagic acid), gallic acid, digalloyl hexoside, galloyl-hexoside, punicalin, pedunculagin, corilagin, 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.

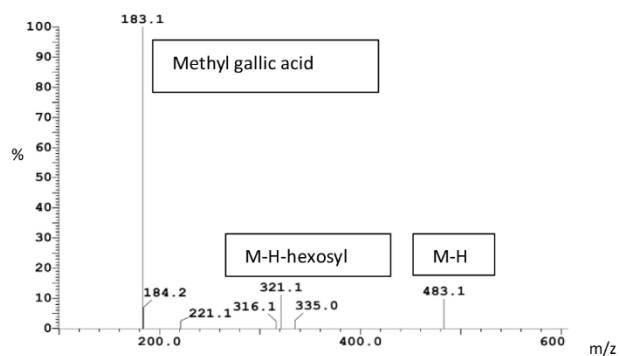
Peak number	Retention time (R _t) (min)	M-H	Fragmentation MS ²	Tentative assignment of the peaks	Chemical class	% Area	Reference
1	9.68	169	169, 125	Gallic acid	Phenolic acid	18.15	(Mena et al. 2012)
2	10.75	483	183, 184, 221, 316 321, 335, 483	Digalloyl hexoside	Phenolic acid derivative	5.55	(Gan et 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)



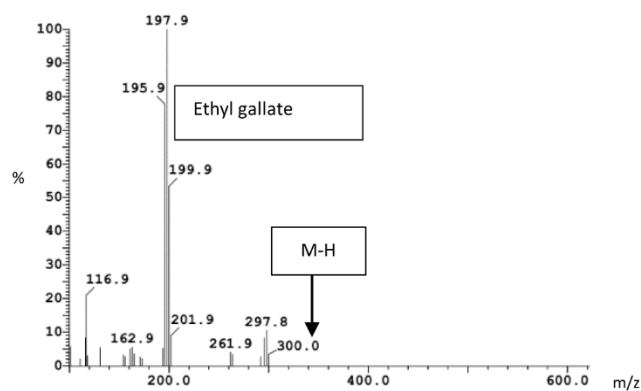
Supplementary Figure 1. Total ion chromatogram (TIC) for PE with Area % for each compound



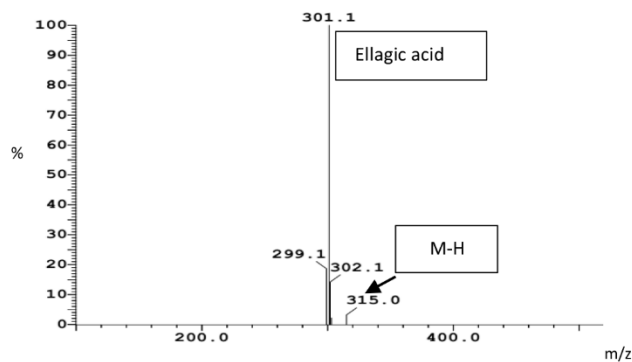
Galloyl hexoside



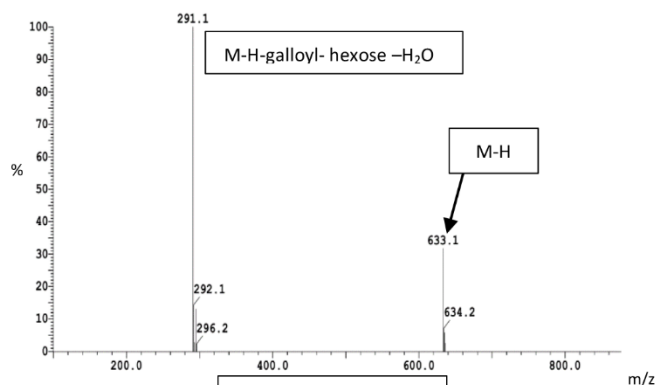
Digalloyl hexoside



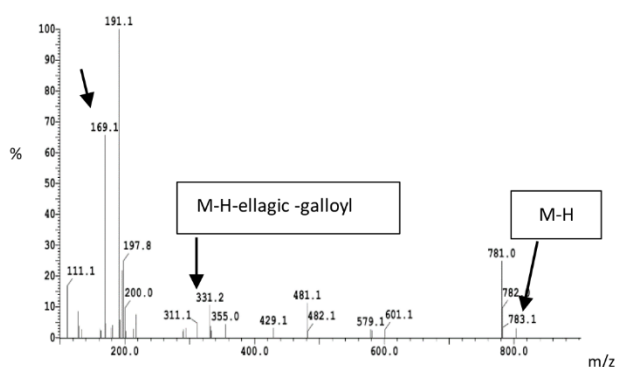
Ellagic acid



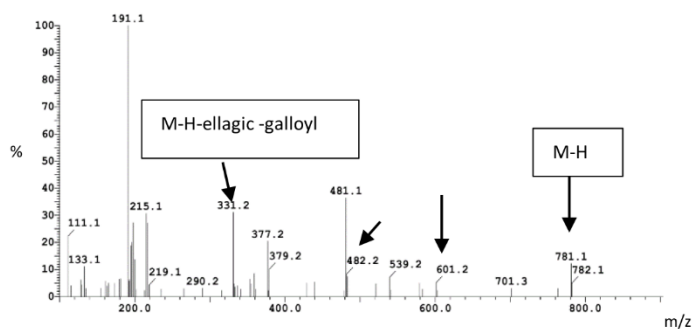
Methyl ellagic acid



Corilagin



Pedunculagin



Punicalin

Supplementary Figure 2. MS² fragmentation for the major identified compounds in PE.

References

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