Electronic Supplementary Material (ESI) for Analytical Methods.

Supporting Information

Sensitive lanthanide label array method for rapid fingerprint analysis of plant polyphenols based on time-resolved luminescence

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UHPLC quantification of phenolic composition of plant samples:

Phenolic content was quantified using Diode-array detector at λ = 280 nm. Monomeric HTs and oligomeric ETs were identified based on their characteristic UV spectrum and MS fragments in UHPLC-DAD-ESI-MS analysis. All these phenolic compounds have been characterized and published earlier from our research group and other groups.[1-12] Published literatures have been used for the identification and characterization of these major polyphenols. Diode-array spectrum and MS chromatogram of each plant sample has been mentioned in **Fig. S1 to S42**. Identified compounds are mentioned with their parent ions and major fragments in the legend of each MS spectrum.

Method development approach:

Method development was carried out in three stages, firstly development phase, then optimization phase and finally the experimental phase. In the development phase, we tested europium (Eu) and terbium (Tb) chelates with different chemistries at different concentrations to find out which chelate is suitable for our purpose. Initially, we tested some plant samples with similar and diverse polyphenol composition at different concentrations. As the plant sample contains a large percentage of sugar, we separated sugar from plant samples using Sep-Pak C18 cartridges (Waters Corporation, USA) and tested the effect of sugar. We didn't find any quenching effect of sugar and there was no change of kinetics in the reaction. To achieve our goal, plant sample concentration and modulator strength should be optimum to get a significant signal and avoiding a very high or low signal. Concentrated sample requires concentrated modulator to quench the signal. Therefore, optimization and finding the best composition was crucial for the development of this method. As we have many parameters to be optimized for fine-tuning the method, we fixed the sample concentration at 0.02 gL^{-1} after initial testing of different concentrations and later

modified the composition of each modulating agent to get optimum signal levels within the detection limit. Each modulator was the combination of chemicals in a certain ratio to introduce unique chemistry. After initial testing with the combination found in the published literature and our own method development experience, we optimized the combination to find the perfect ratio of chemicals for each modulator to get signal within detection limit for plant samples at the concentration of 0.02 gL^{-1}. During the optimization phase, many experiments were conducted with different ratios of chemicals of modulators to find the optimum ratio. Each modulator has its unique chemistry which allows quenching the sample differently. Therefore, we started with a certain ratio and then each of them was modified to achieve optimum signal. Once we optimized it for plant samples, then this combination can be used for all plant species to reveal fingerprints.

References:

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4 A. Tuominen, E. Toivonen, P. Mutikainen, J. P. Salminen, *Phytochemistry*, 2013, **95**, 394–407.

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7. J. Moilanen, J. P. Salminen, *Chemoecology*, 2008, **18**, 73–83.

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Sample 1 (*Acer platanoides* L.)

Phenolic content	143652
Oligomeric ET	0
Monomeric HT	88520
% of oligomeric ETs	0
% of monomeric HTs	61.62
% of HTs	61.62

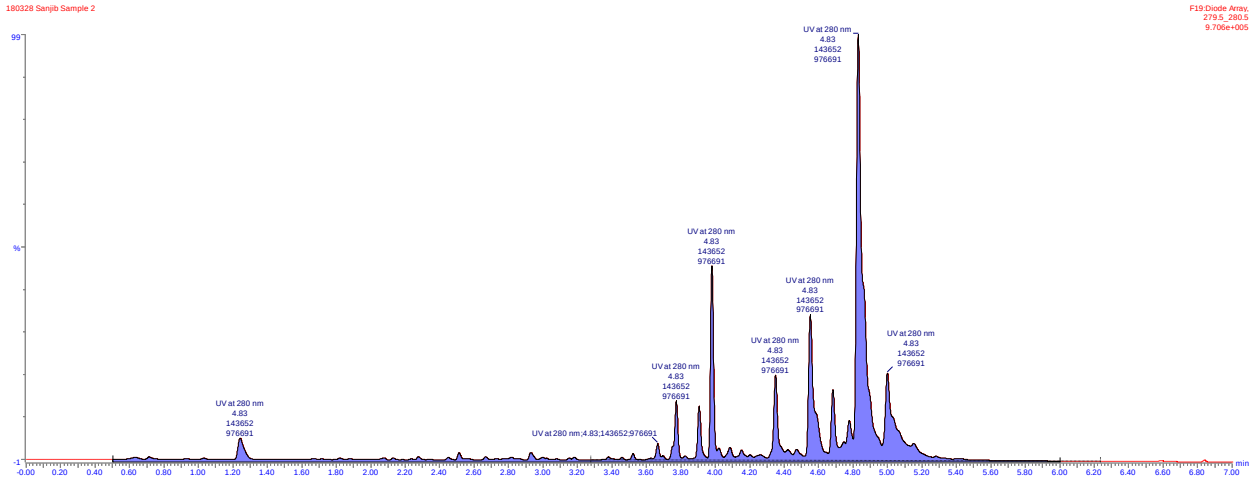

Fig. S1: UHPLC quantification of total phenolic content by Diode-array detector at λ= 280 nm.

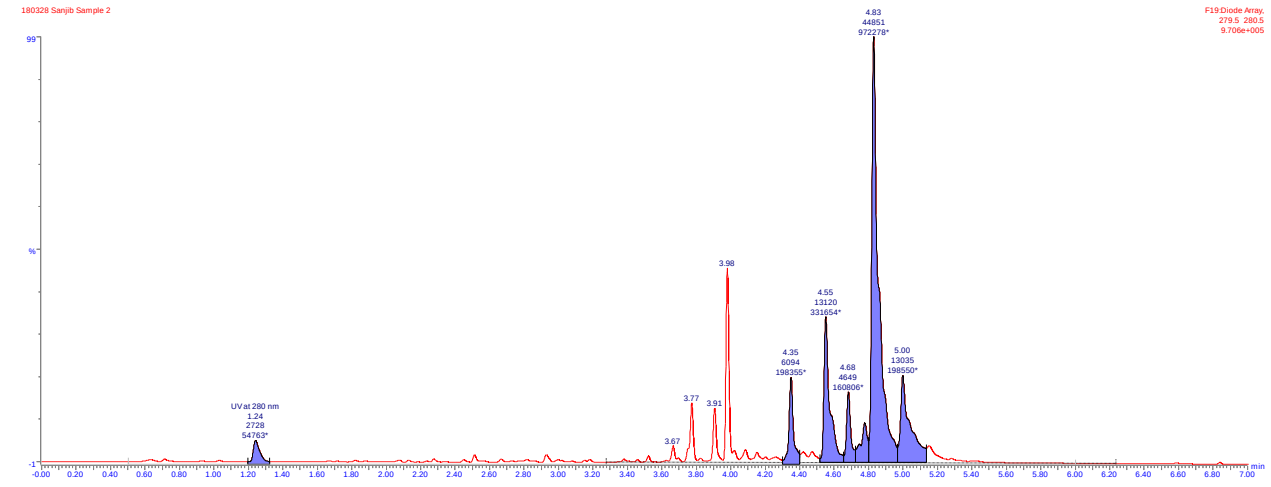

Fig. S2: UHPLC quantification of Monomeric HTs by Diode-array detector at λ= 280 nm.

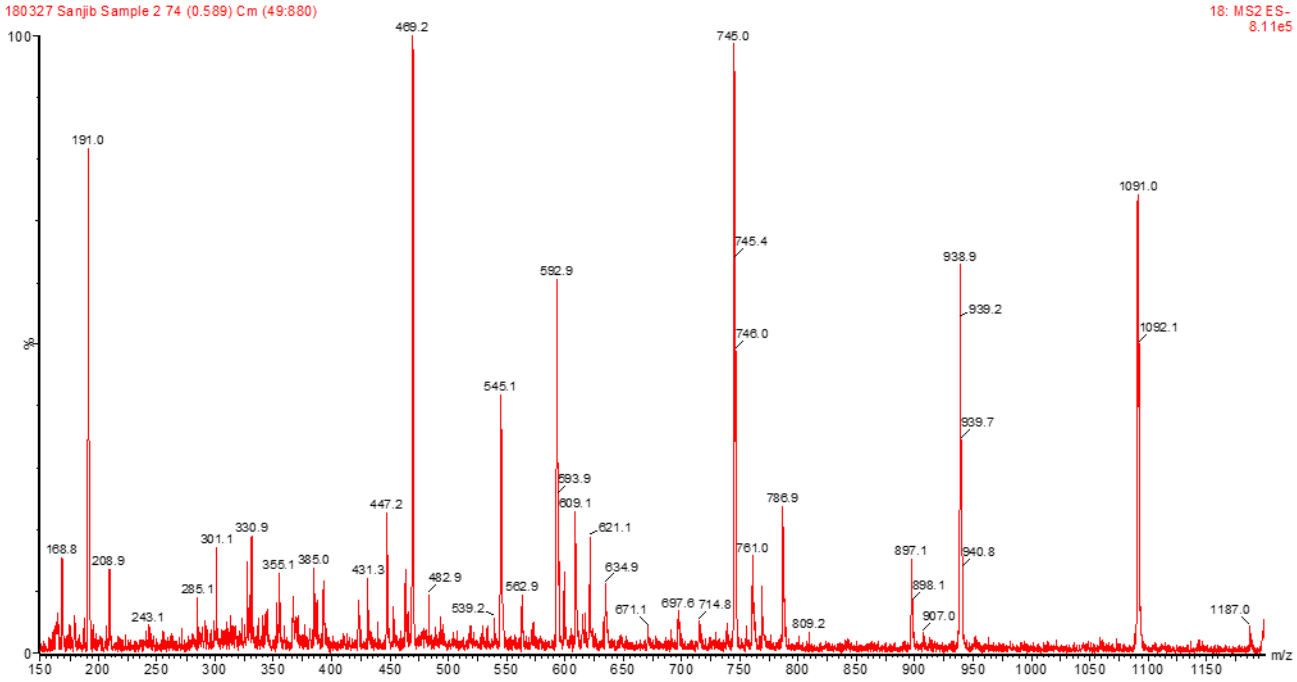

Fig. S3: MS spectrum from *m/z* 150 to 1200. ESI-MS identification: **hexagalloyl glucose** *m/z* at 1091 $[M–H]^{–}$; **heptagallyol glucose** *m/z* at 621 $[M–2H]^{2–}$; **octagallyol glucose** *m/z* at 697 $[M–2H]^{2–}$.[3,5]

Sample 2 (*Geranium sylvaticum* L.)

Phenolic content	389903
Oligomeric ET	0
Monomeric HT	259829
% of oligomeric ETs	0
% of monomeric HTs	66.64
% of HTs	66.64

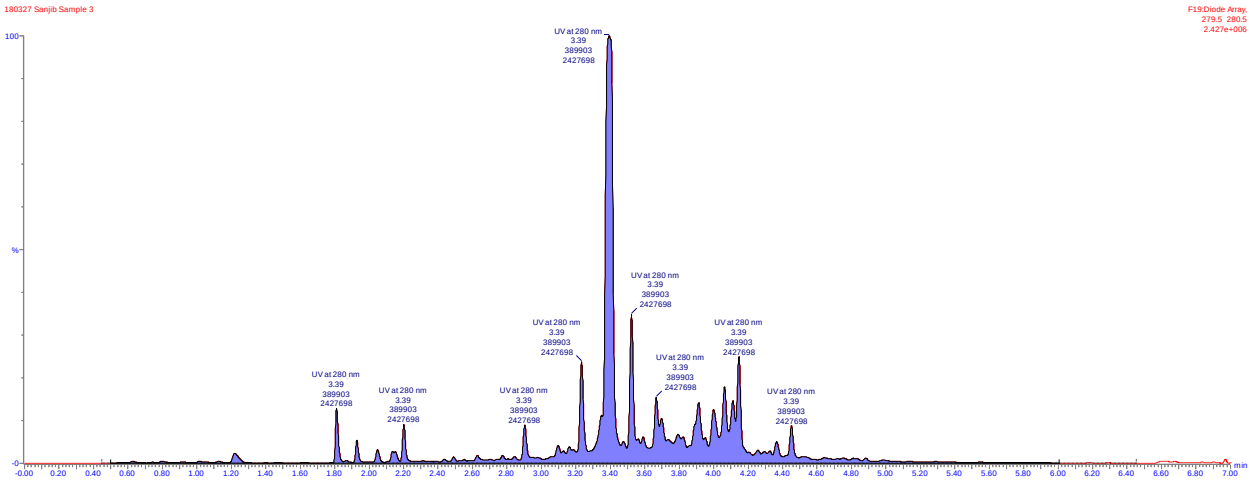

Fig. S4: UHPLC quantification of total phenolic content by Diode-array detector at λ= 280 nm.

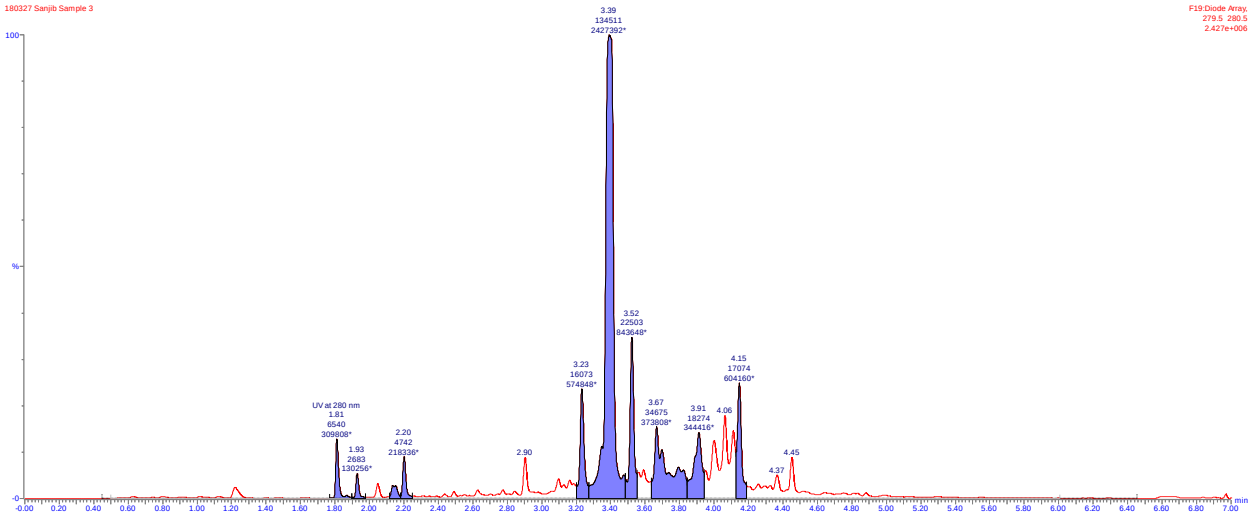

Fig. S5: UHPLC quantification of Monomeric HTs by Diode-array detector at λ= 280 nm.

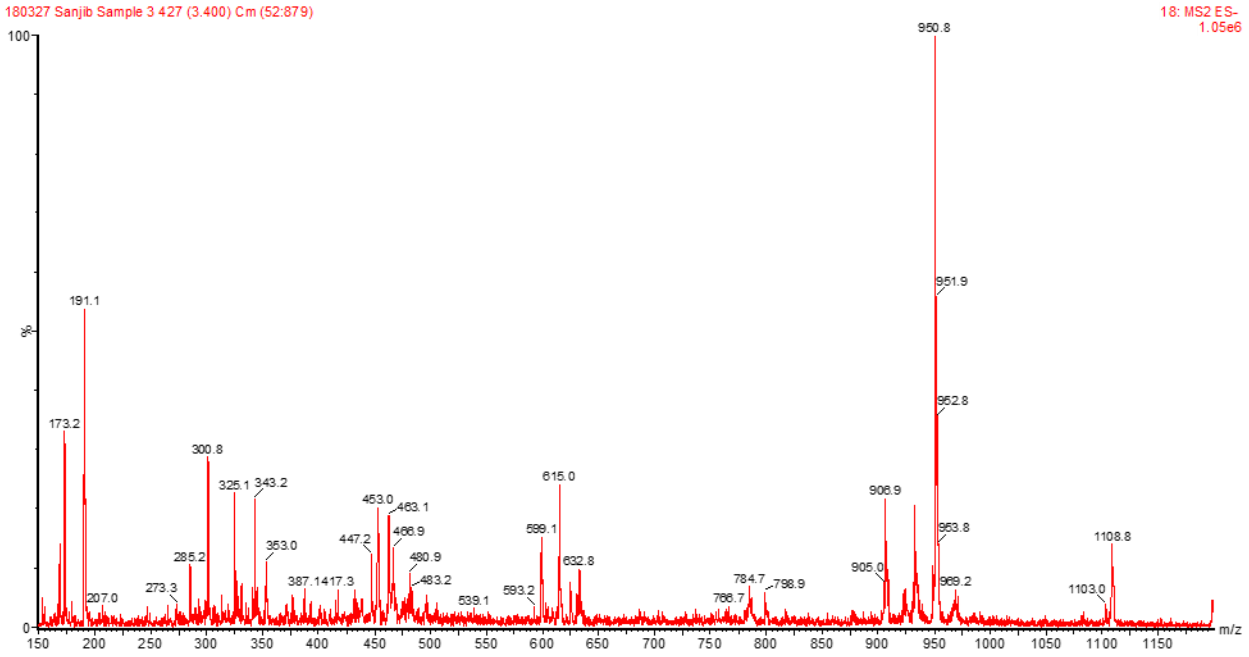

Fig. S6: MS spectrum from *m/z* 150 to 1200. ESI-MS identification: **Geraniin** *m/z* at 951 [M–H]$^{-}$, *m/z* at 933 [M–H_2O–H]$^{-}$, *m/z* at 301 [ellagic acid–H]$^{-}$; **Ascorgeraniin** *m/z* at 1109 [M–H]$^{-}$.[4]

Sample 3 (*Terminalia chebula* Retz.)

Phenolic content	182403
Oligomeric ET	0
Monomeric HT	126560
% of oligomeric ETs	0
% of monomeric HTs	69.38
% of HTs	69.38

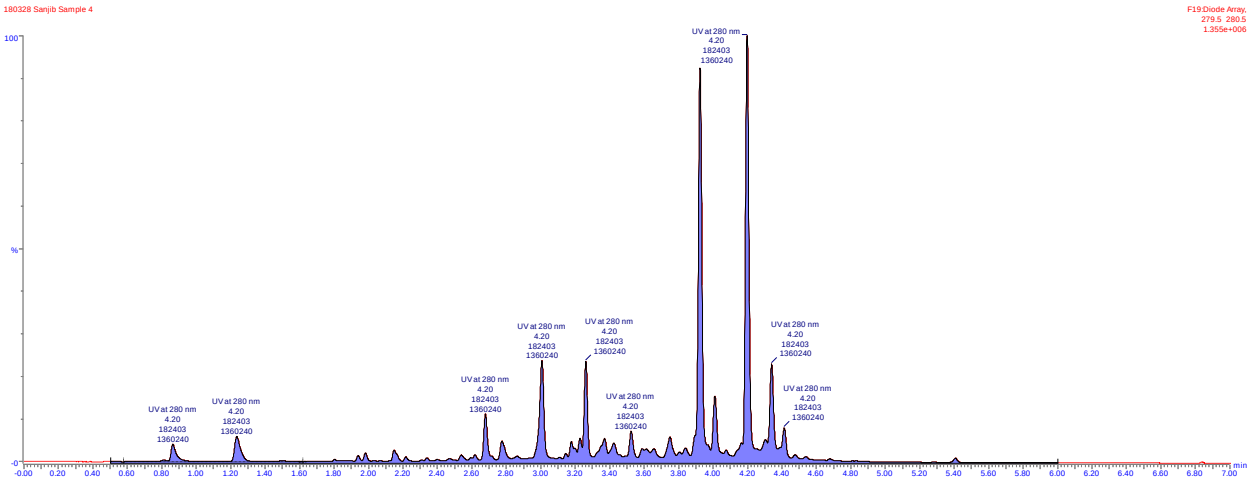

Fig. S7: UHPLC quantification of total phenolic content by Diode-array detector at λ= 280 nm.

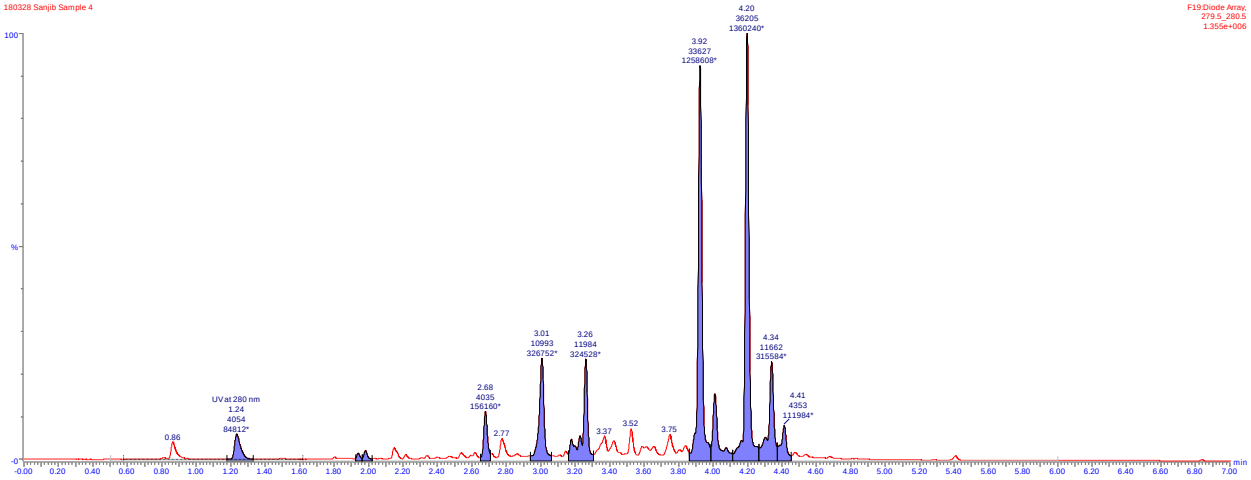

Fig. S8: UHPLC quantification of Monomeric HTs by Diode-array detector at λ= 280 nm.

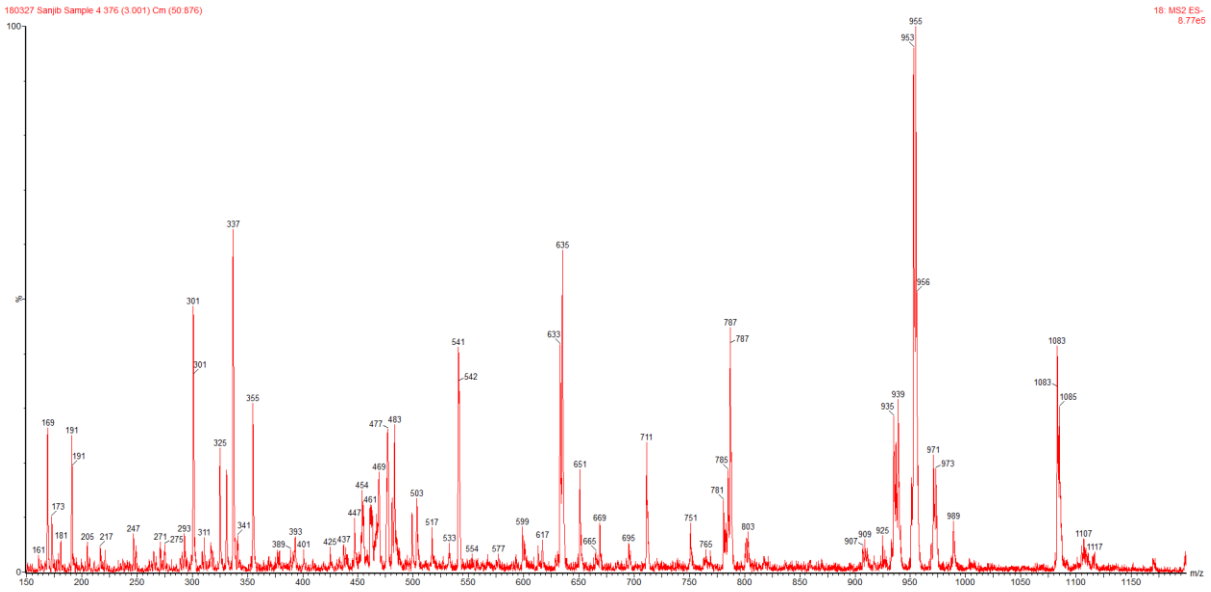

Fig. S9: MS spectrum from *m/z* 150 to 1200. ESI-MS identification: **Chebulagic acid** *m/z* at 955 $[M–H]^-$; **Chebulanin** *m/z* at 651 $[M–H]^-$; **Chebulinic acid** *m/z* at 955 $[M–H]^-$.[6]

Sample 4 (*Filipendula ulmaria* (L.) Maxim.)

Phenolic content	703589
Oligomeric ET	126695
Monomeric HT	287263
% of oligomeric ETs	18
% of monomeric HTs	40.83
% of HTs	58.83

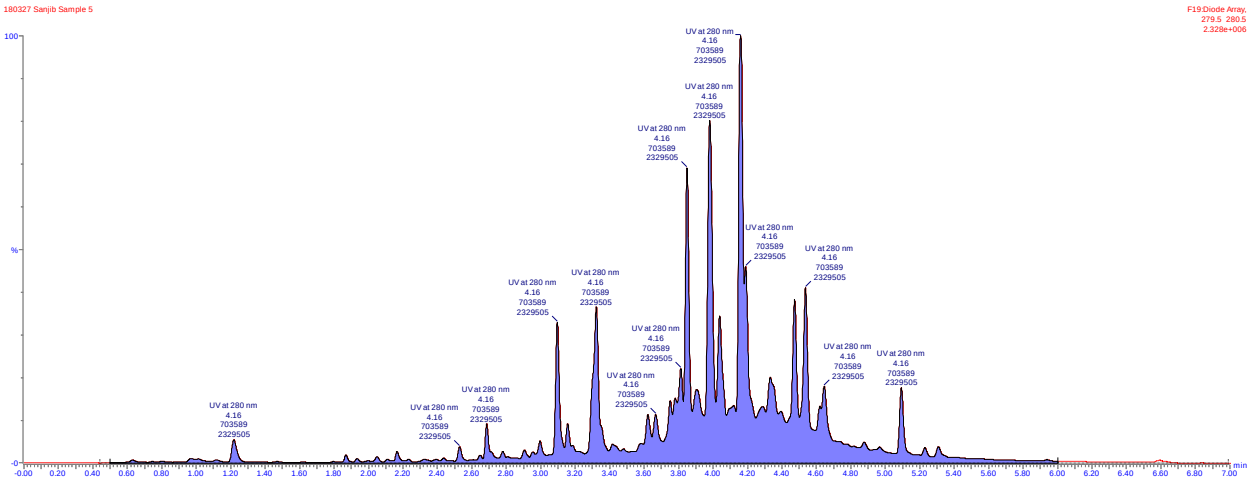

Fig. S10: UHPLC quantification of total phenolic content by Diode-array detector at λ= 280 nm.

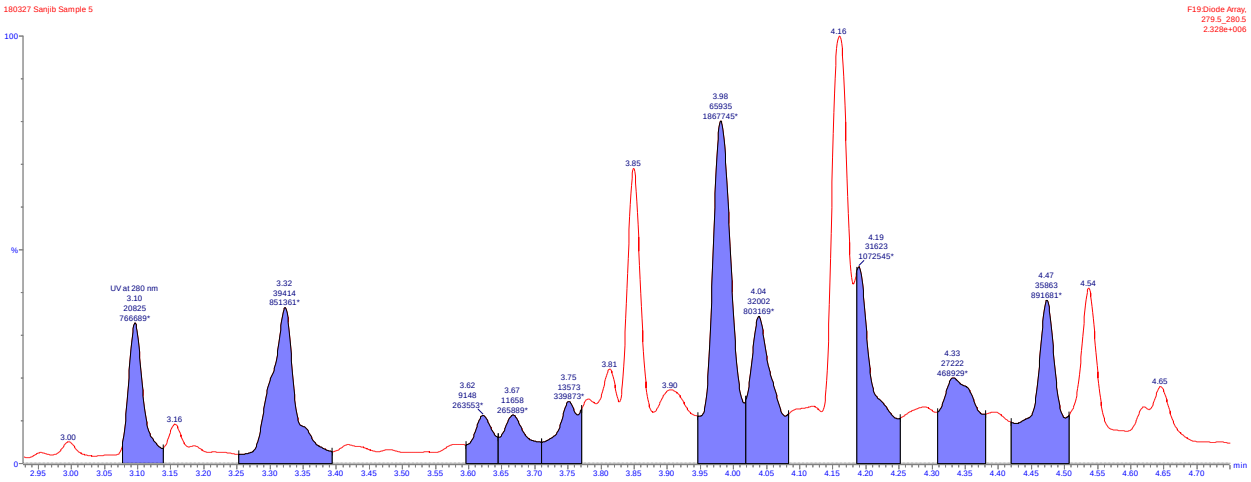

Fig. S11: UHPLC quantification of Monomeric HTs by Diode-array detector at λ= 280 nm.

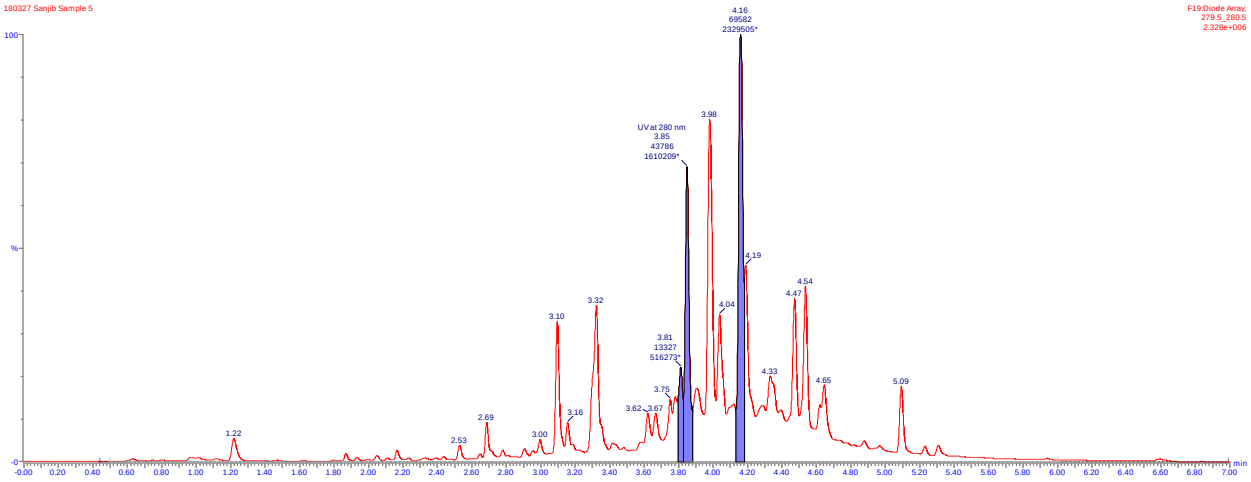

Fig. S12: UHPLC quantification of Oligomeric ETs by Diode-array detector at λ= 280 nm

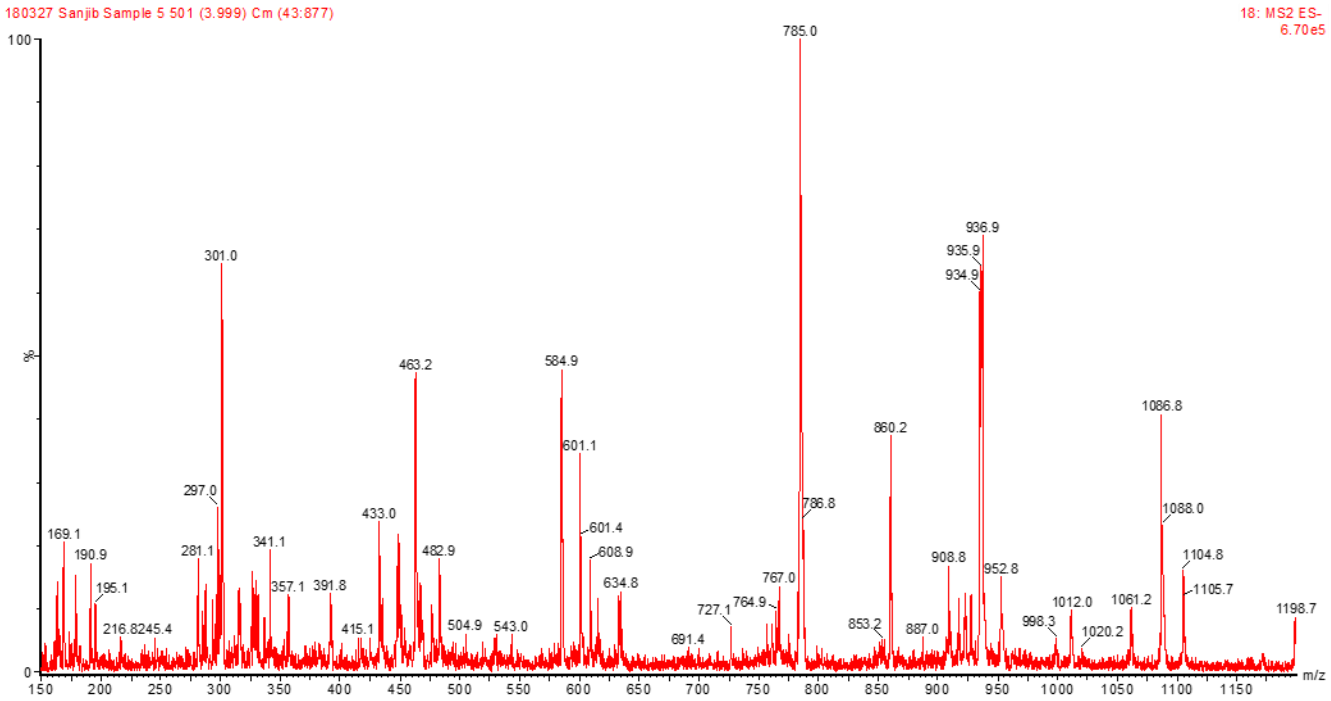

Fig. S13: MS spectrum from *m/z* 150 to 1200. ESI-MS identification: **Tellimagrandin I** *m/z* at 785 $[M–H]^-$; **Tellimagrandin II** *m/z* at 937 $[M–H]^-$, *m/z* at 301 [ellagic acid–H]$^-$; **Casuarictin** *m/z* at 935 $[M–H]^-$; **Isostrictinin** *m/z* at 633 $[M–H]^-$; **Pedunculagin** *m/z* at 783 $[M–H]^-$, *m/z* at 391 $[M–2H]^{2-}$.[2,5,7]

Sample 5 (*Punica granatum* L.)

Phenolic content	169154
Oligomeric ET	12577
Monomeric HT	12360
% of oligomeric ETs	7.43
% of monomeric HTs	7.31
% of HTs	14.74

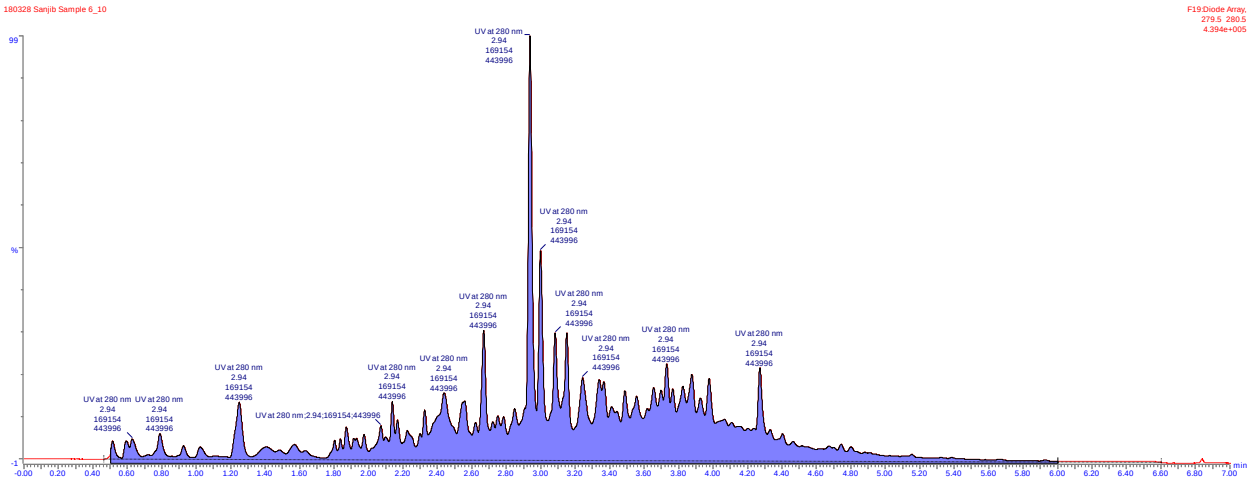

Fig. S14: UHPLC quantification of total phenolic content by Diode-array detector at λ= 280 nm.

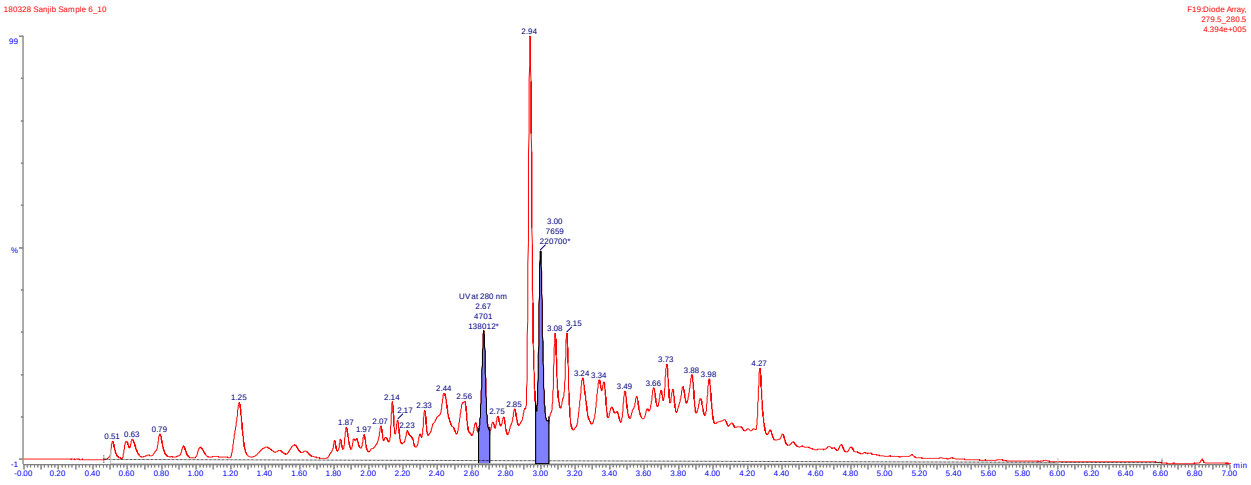

Fig. S15: UHPLC quantification of Monomeric HTs by Diode-array detector at λ= 280 nm.

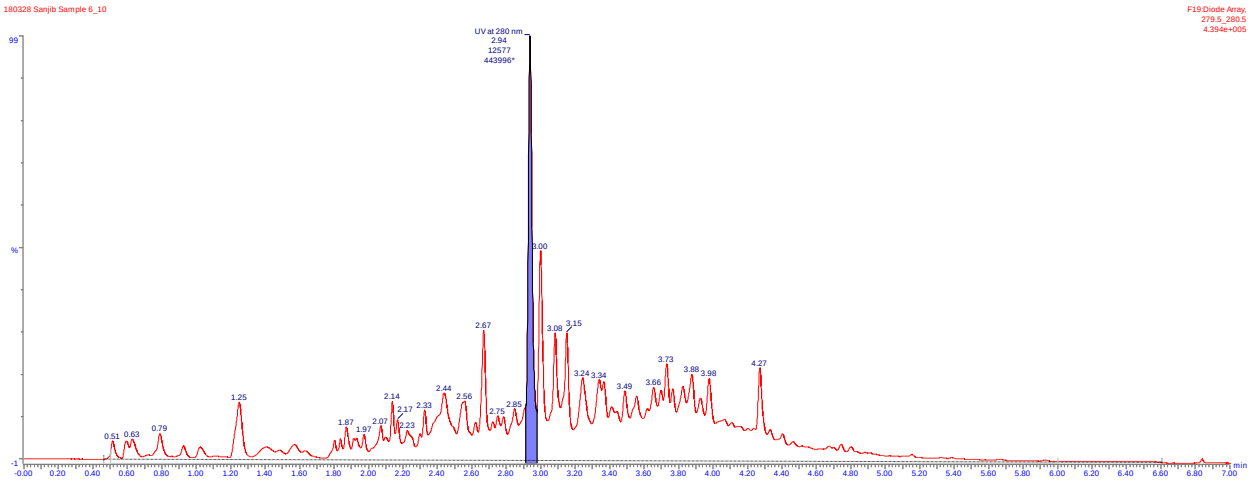

Fig. S16: UHPLC quantification of Oligomeric ETs by Diode-array detector at λ= 280 nm.

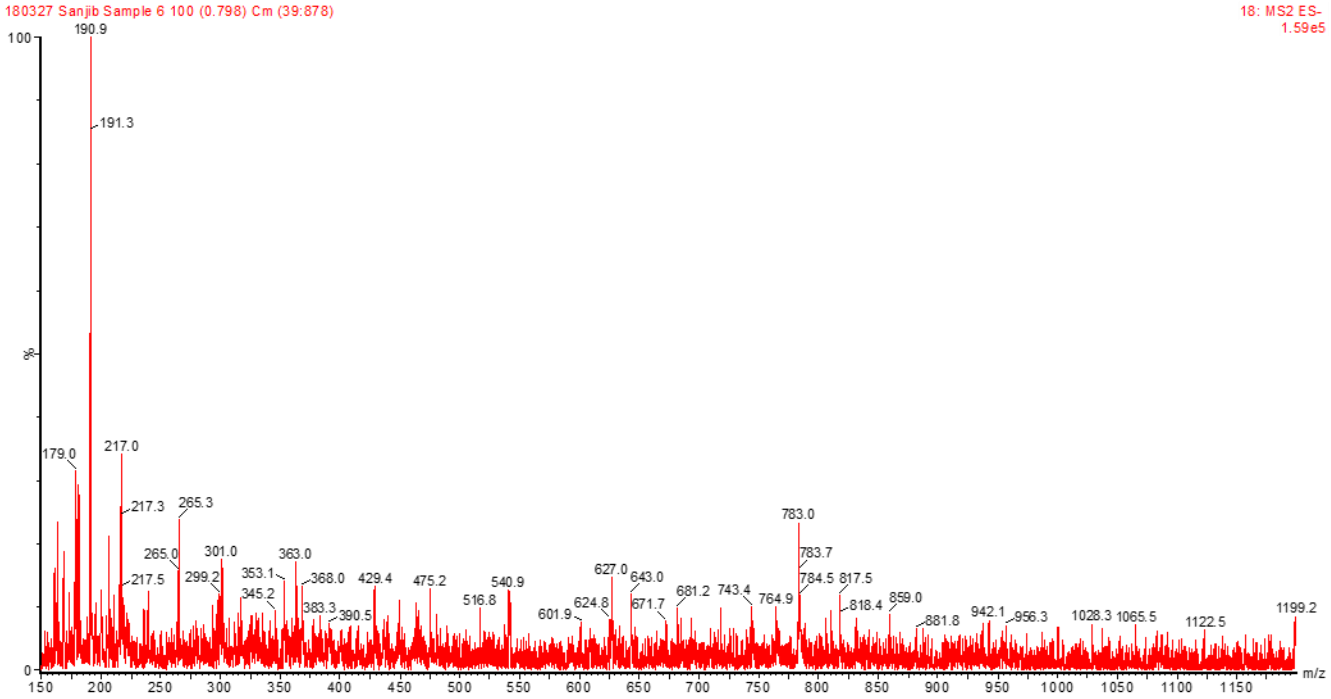

Fig. S17: MS spectrum from *m/z* 150 to 1200. ESI-MS identification: **Punicalagin** *m/z* at 1065 $[M–H_2O–H]^–$.[8]

Sample 6 (*Hippophaë rhamnoides* L.)

Phenolic content	224678
Oligomeric ET	0
Monomeric HT	129382
% of oligomeric ETs	0
% of monomeric HTs	57.59
% of HTs	57.59

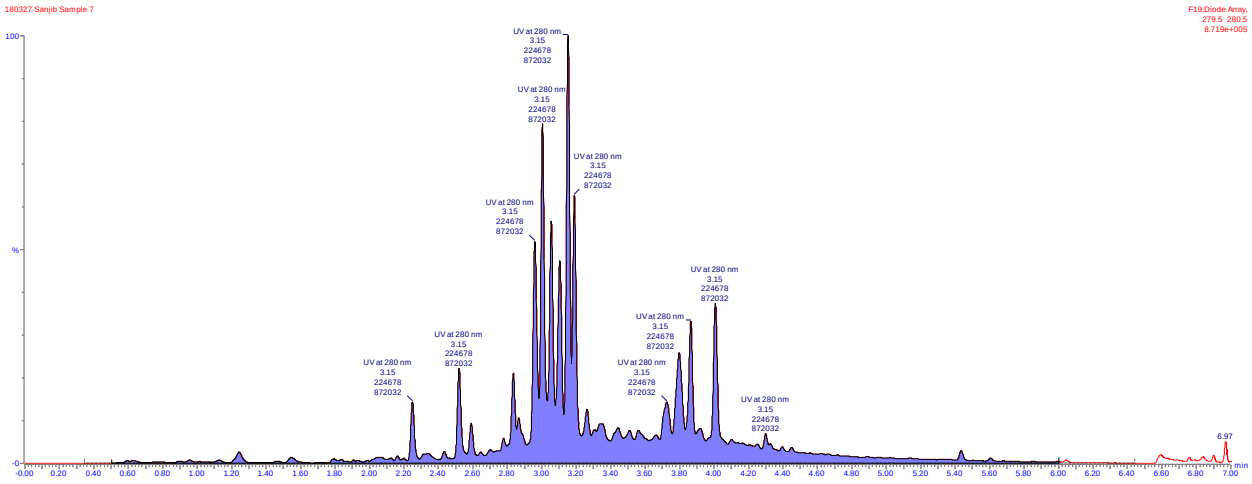

Fig. S18: UHPLC quantification of total phenolic content by Diode-array detector at λ= 280 nm.

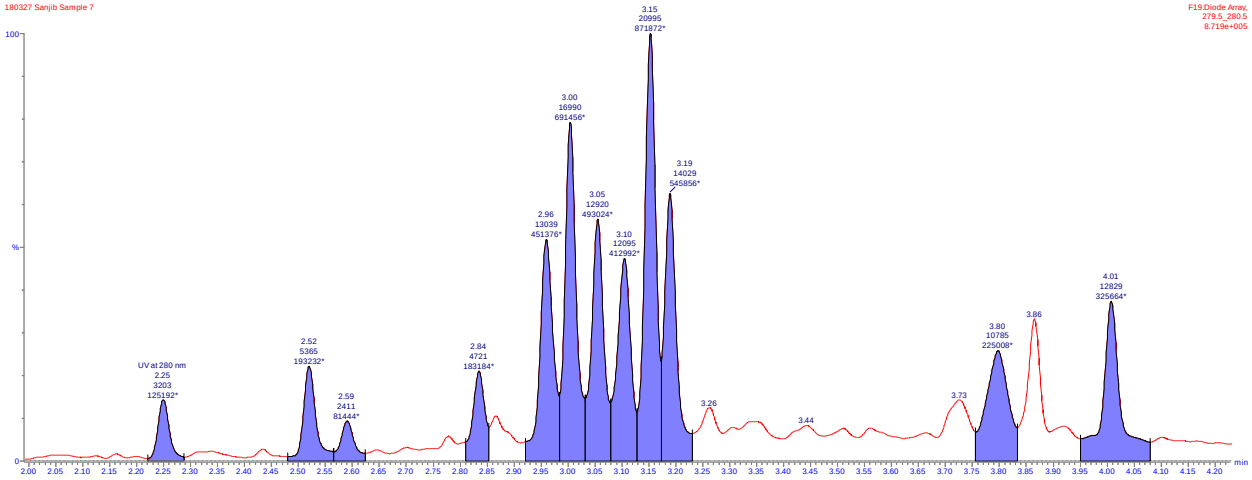

Fig. S19: UHPLC quantification of Monomeric HTs by Diode-array detector at λ= 280 nm.

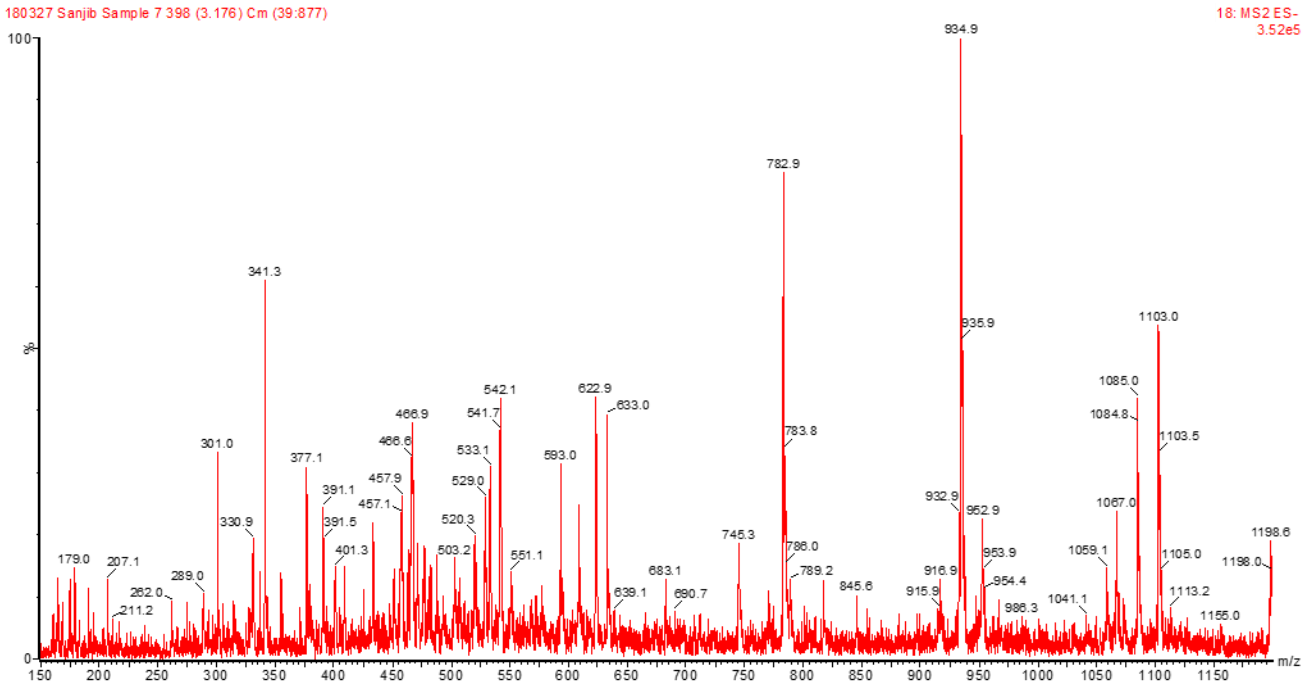

Fig. S20: MS spectrum from *m/z* 150 to 1200. ESI-MS identification: **Vescalagin** *m/z* at 933 $[M–H]^-$, *m/z* at 915 $[M–H_2O–H]^-$, *m/z* at 466 $[M–2H]^{2-}$, *m/z* at 457 $[M–H_2O –2H]^{2-}$, *m/z* at 301 [ellagic acid–H]$^-$; **Castalagin** *m/z* at 933 $[M–H]^-$, *m/z* at 466 $[M–2H]^{2-}$, *m/z* at 301 [ellagic acid–H]$^-$; **Vescavaloninic** acid *m/z* at 1101 $[M–H]^-$, *m/z* at 1083 $[M–H_2O–H]^-$, *m/z* at 528 [M–COOH– 2H]$^{2-}$, *m/z* at 519 [$[M–H_2O–COOH–2H]^{2-}$, *m/z* at 301 [ellagic acid–H]$^-$; **Castavaloninic acid** *m/z* at 1101 $[M–H]^-$, *m/z* at 528 $[M–COOH–2H]^{2-}$; **Stachyurin** *m/z* at 935 $[M–H]^-$; **Casuarinin** *m/z* at 935 $[M–H]^-$.[2,7,9,10]

Sample 7 (*Geum rivale* L.)

Phenolic content	73357
Oligomeric ET	27185
Monomeric HT	5629
% of oligomeric ETs	37.06
% of monomeric HTs	7.67
% of HTs	44.73

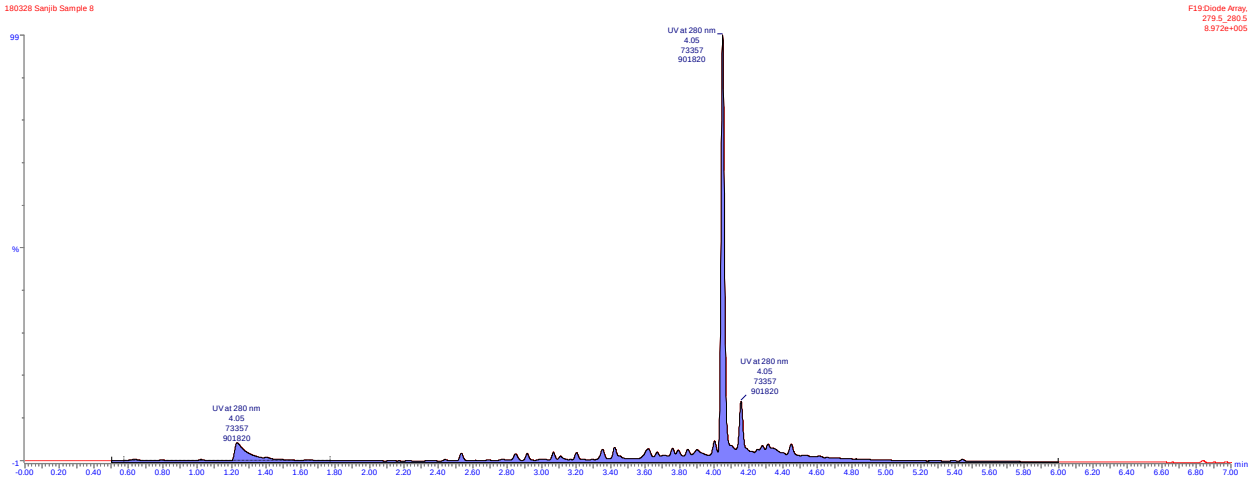

Fig. S21: UHPLC quantification of total phenolic content by Diode-array detector at λ= 280 nm.

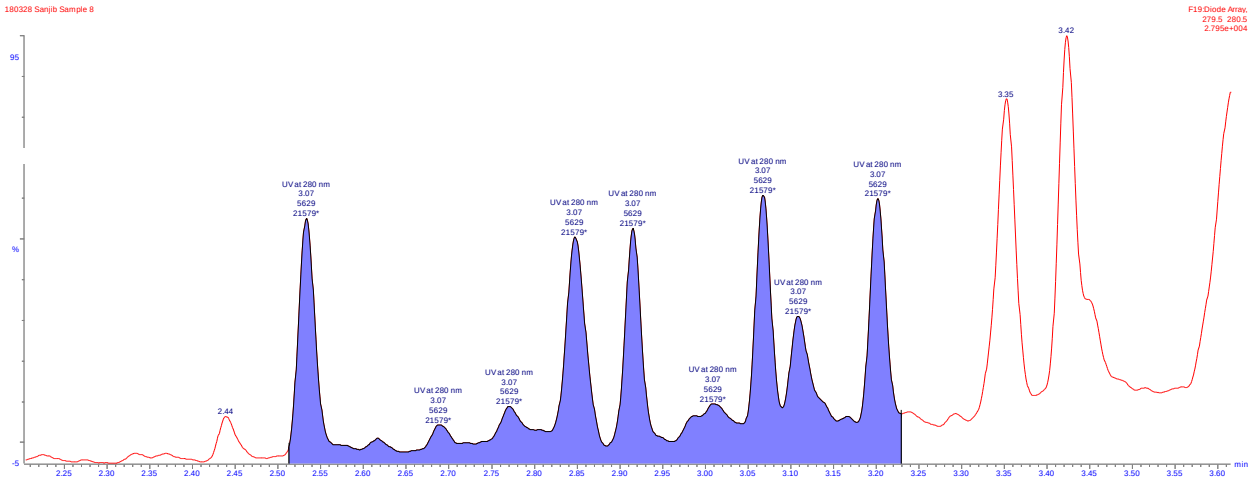

Fig. S22: UHPLC quantification of Monomeric HTs by Diode-array detector at λ= 280 nm.

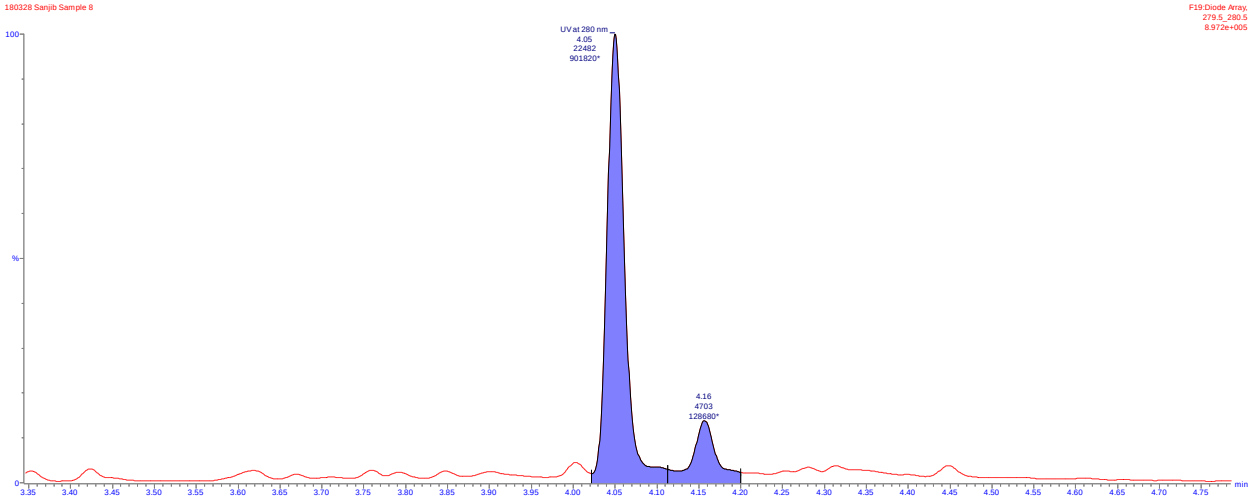

Fig. S23: UHPLC quantification of Oligomeric ETs by Diode-array detector at λ= 280 nm.

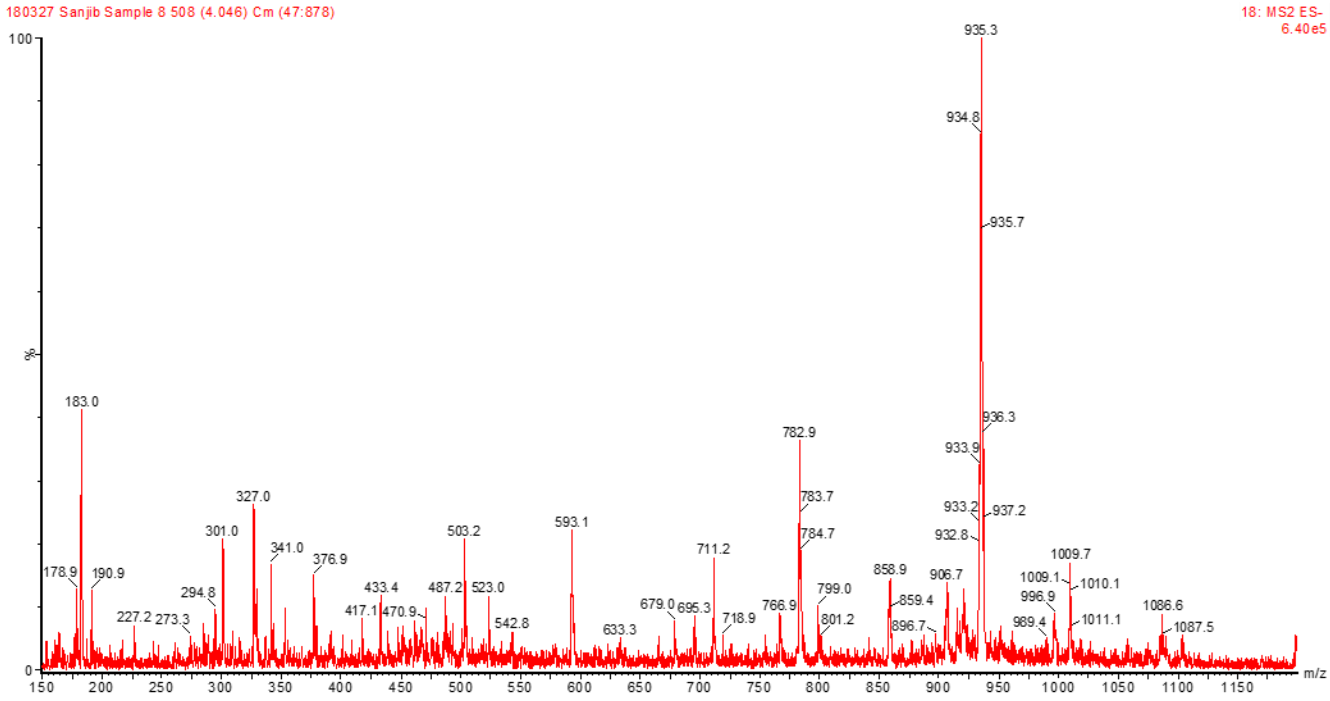

Fig. S24: MS spectrum from *m/z* 150 to 1200. ESI-MS identification: **Rugosin D** *m/z* at 936 [M–2H] $^{2-}$; **Rugosin E** *m/z* at 860 [M–2H]$^{2-}$.[11]

Sample 8 (*Fragaria vesca* L.)

Phenolic content	311205
Oligomeric ET	52383
Monomeric HT	22671
% of oligomeric ETs	16.83
% of monomeric HTs	7.28
% of HTs	24.11

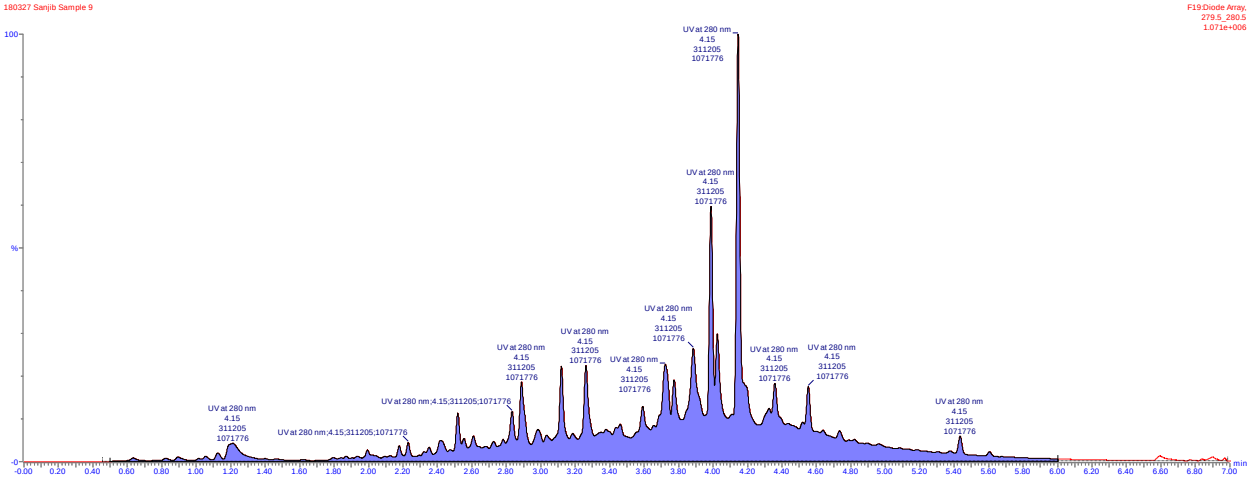

Fig. S25: UHPLC quantification of total phenolic content by Diode-array detector at λ= 280 nm.

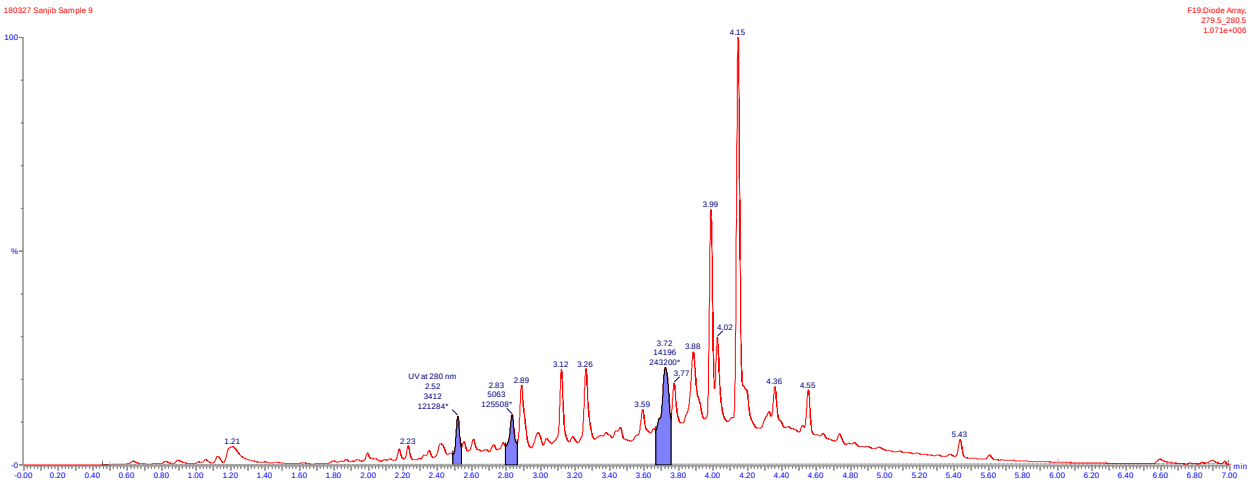

Fig. S26: UHPLC quantification of Monomeric HTs by Diode-array detector at λ= 280 nm.

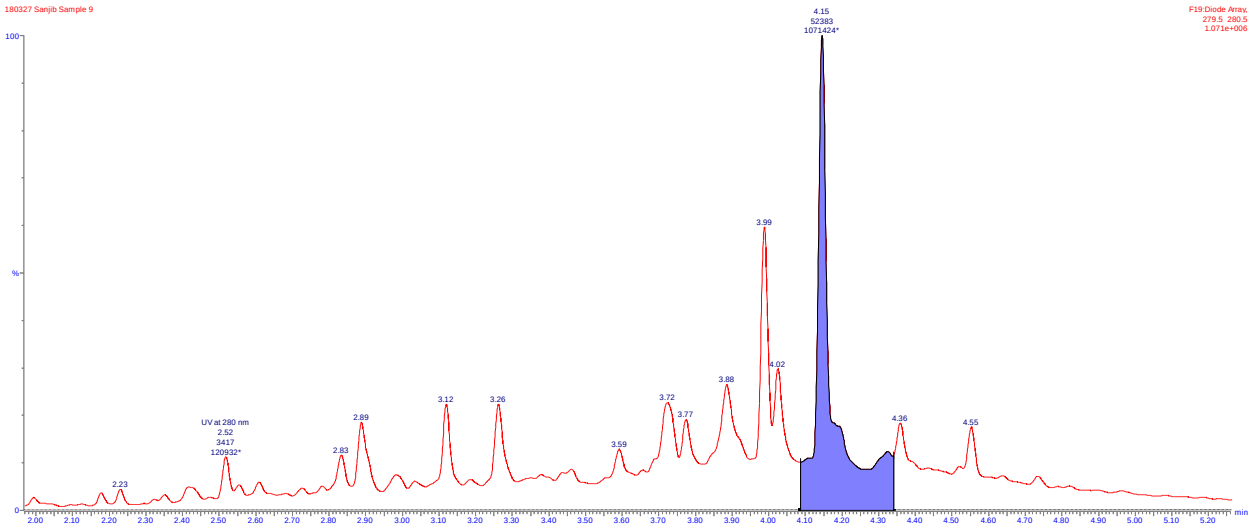

Fig. S27: UHPLC quantification of Oligomeric ETs by Diode-array detector at λ= 280 nm.

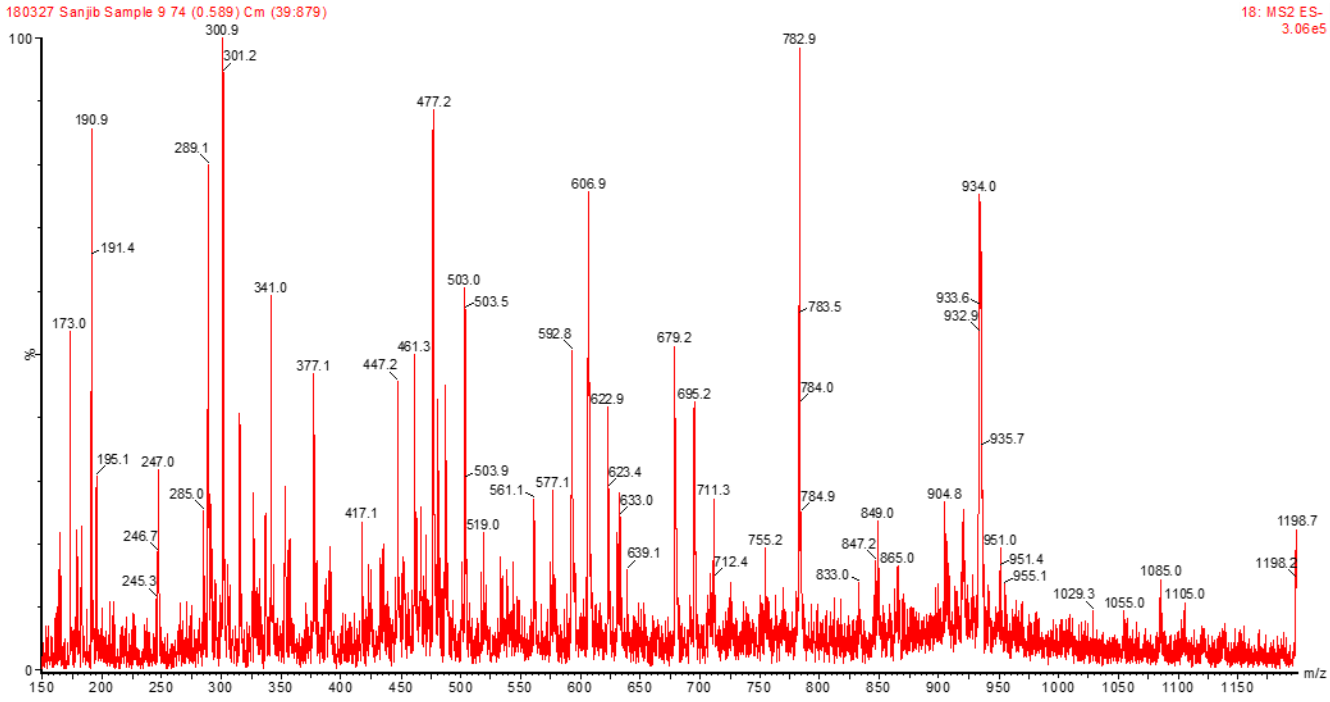

Fig. S28: MS spectrum from *m/z* 150 to 1200. ESI-MS identification: **Agrimoniin** *m/z* at 934 $[M–2H]^{2–}$, m/z at 301 [ellagic acid–H]$^{–}$; **Gemin A** *m/z* at 935 $[M–2H]^{2–}$, *m/z* at 301 [ellagic acid–H]$^{–}$.[2,7]

Sample 9 (*Epilobium angustifolium (L.) Scop.*)

Phenolic content	106672
Oligomeric ET	42705
Monomeric HT	0
% of oligomeric ETs	40.03
% of monomeric HTs	0
% of HTs	40.03

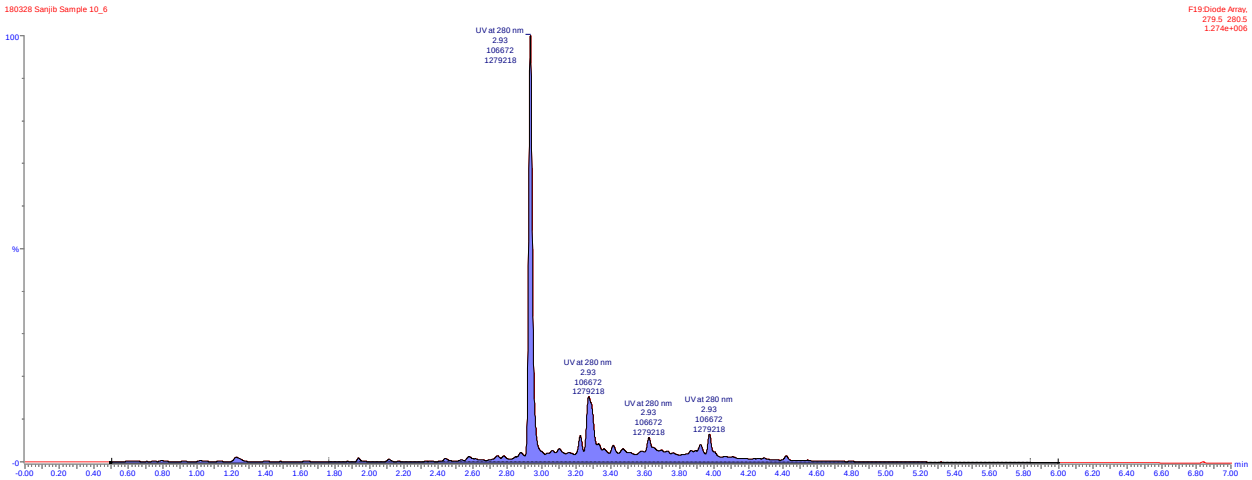

Fig. S29: UHPLC quantification of total phenolic content by Diode-array detector at λ= 280 nm.

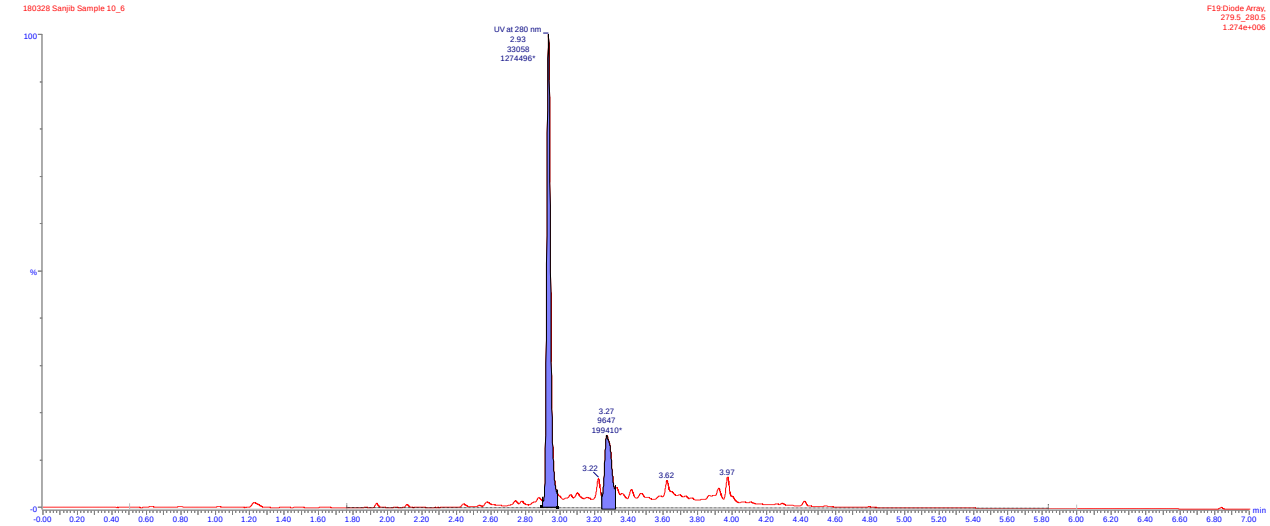

Fig. S30: UHPLC quantification of Oligomeric ETs by Diode-array detector at λ= 280 nm.

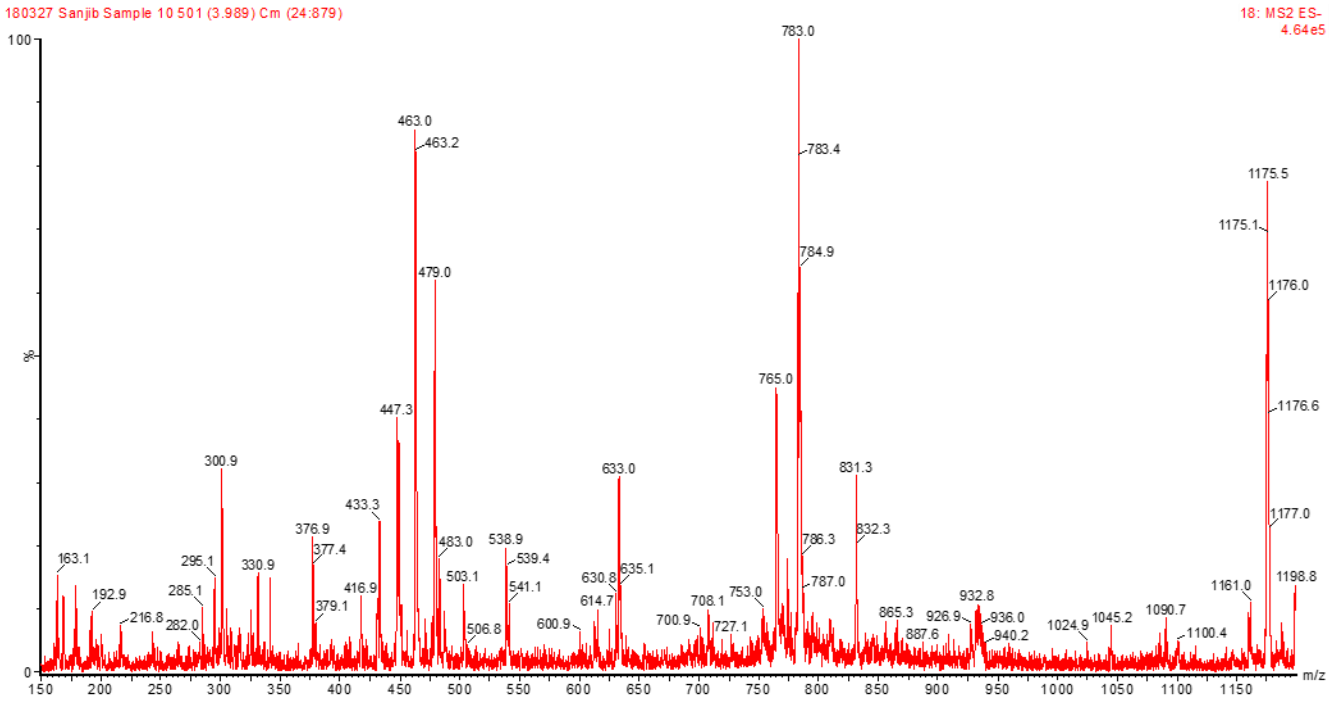

Fig. S31: MS spectrum from *m/z* 150 to 1200. ESI-MS identification: **Oenothein A** *m/z* at 1175 $[M–2H]^{2–}$; **Oenothein B** *m/z* at 783 $[M–2H]^{2-}$; **Tellimagrandin I** *m/z* at 785 $[M–H]^{–}$; **Tellimagrandin II** *m/z* at 937 $[M–H]^{–}$, *m/z* at 301 [ellagic acid–H]$^{–}$.[2,7,12]

Sample 10 (*Rubus idaeus* L.)

Phenolic content	503367
Oligomeric ET	196626
Monomeric HT	0
% of oligomeric ETs	39.06
% of monomeric HTs	0
% of HTs	39.06

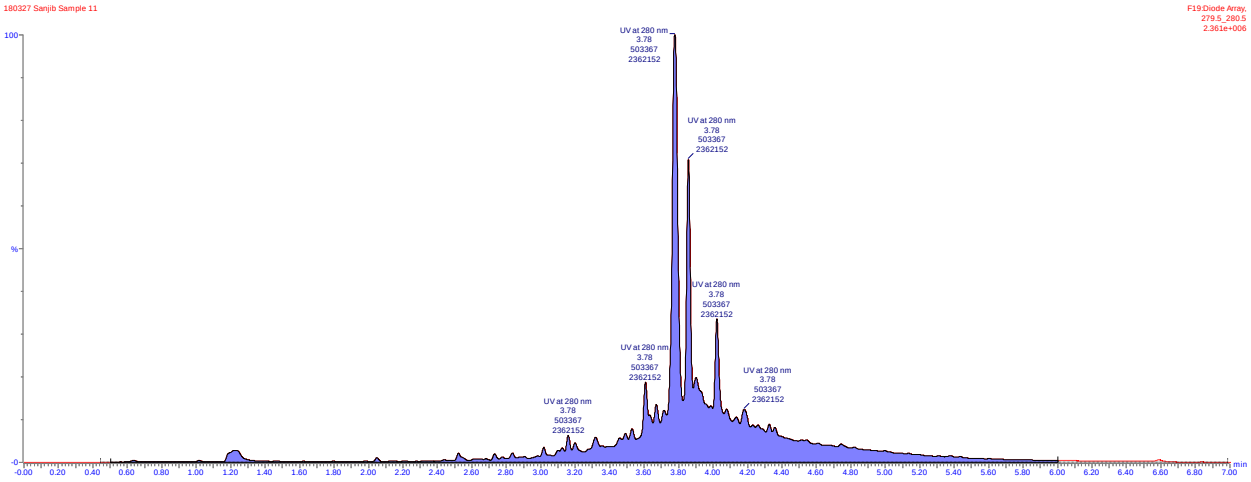

Fig. S32: UHPLC quantification of total phenolic content by Diode-array detector at λ= 280 nm.

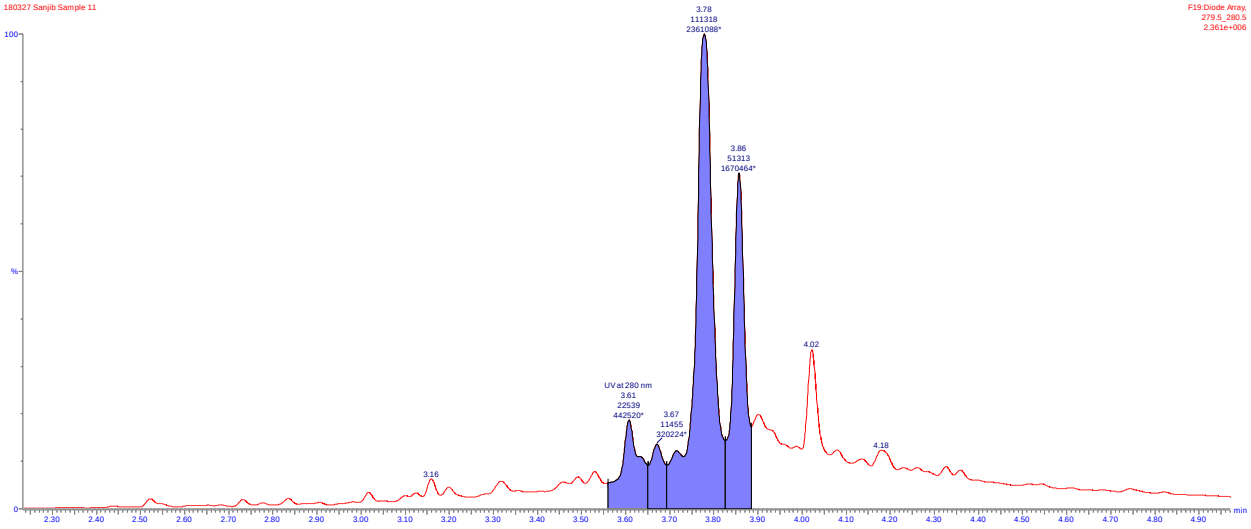

Fig. S33: UHPLC quantification of Oligomeric ETs by Diode-array detector at λ= 280 nm.

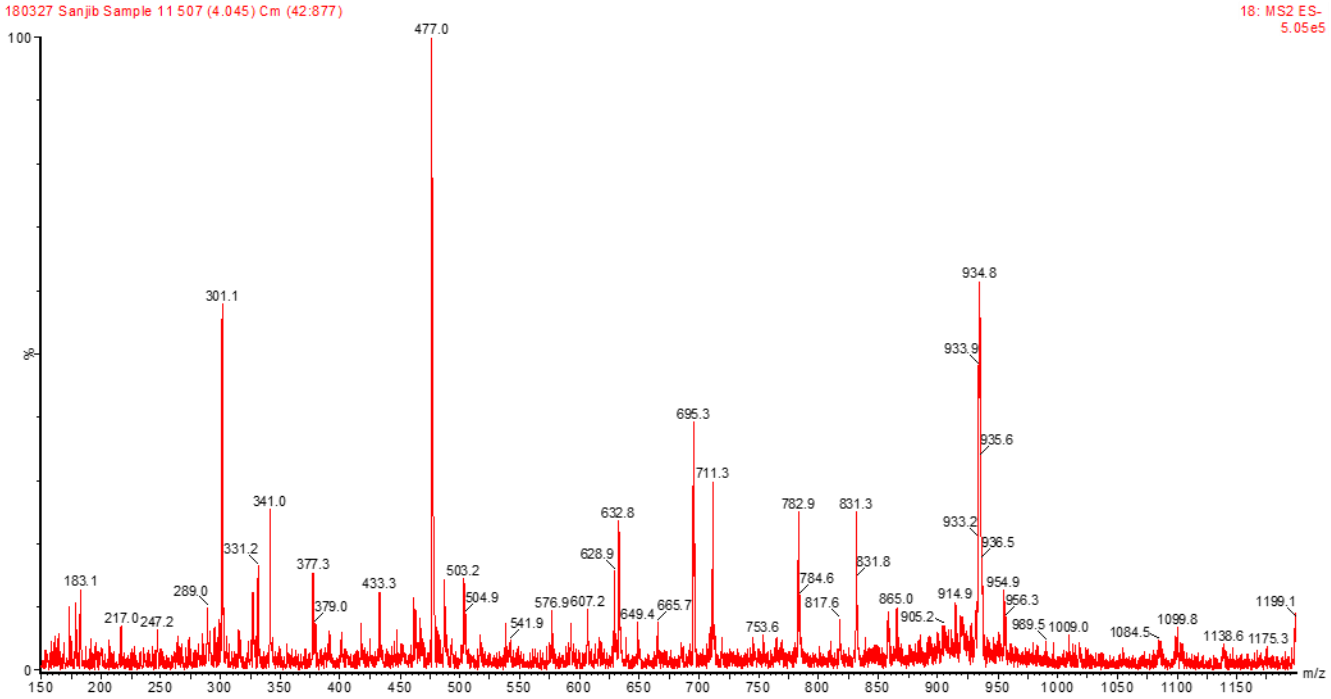

Fig. S34: MS spectrum from *m/z* 150 to 1200. ESI-MS identification: **Sanguiin H-6** *m/z* at 934 $[M–2H]^{2–}$, *m/z* at 301 $[ellagic\ acid–H]^{–}$; **Lambertianin C** *m/z* at 934 $[M–3H]^{3–}$, *m/z* at 301 $[ellagic\ acid–H]^{–}$.[7]

Sample 11 (*Quercus robur* L.)

Phenolic content	315126
Oligomeric ET	36243
Monomeric HT	101039
% of oligomeric ETs	11.50
% of monomeric HTs	32.06
% of HTs	43.56

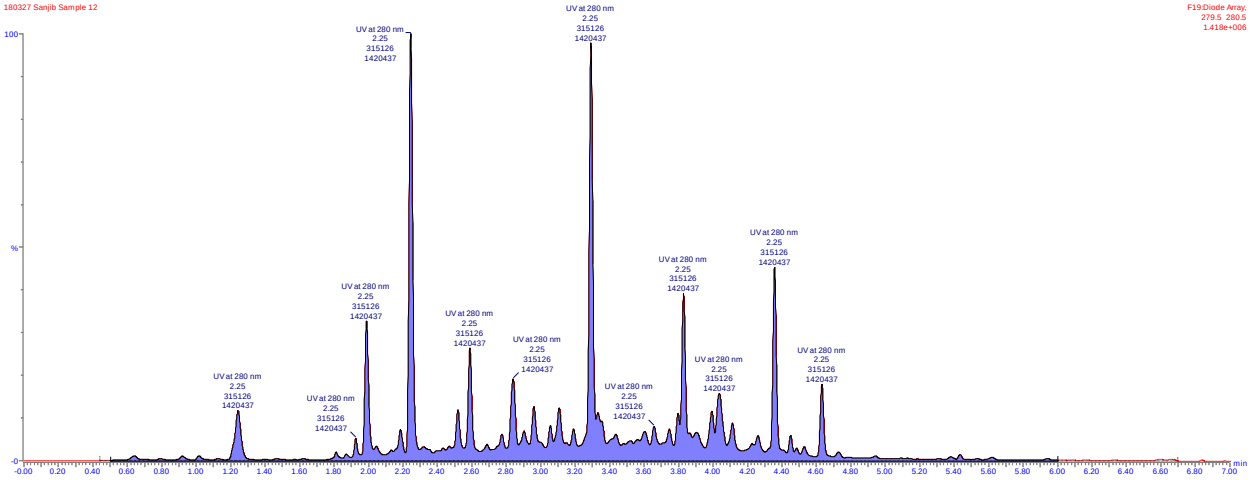

Fig. S35: UHPLC quantification of total phenolic content by Diode-array detector at λ= 280 nm.

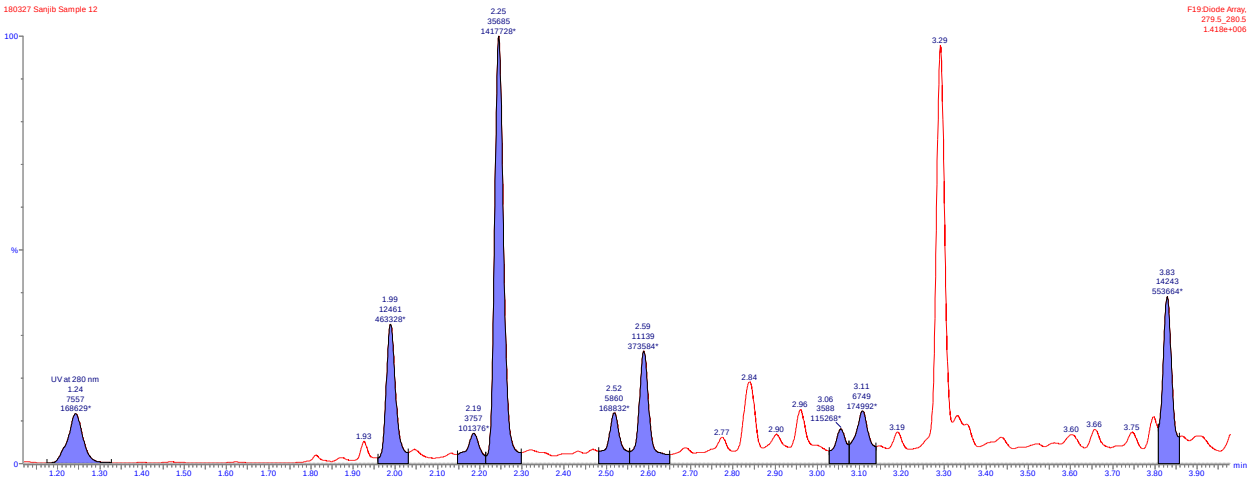

Fig. S36: UHPLC quantification of Monomeric HTs by Diode-array detector at λ= 280 nm.

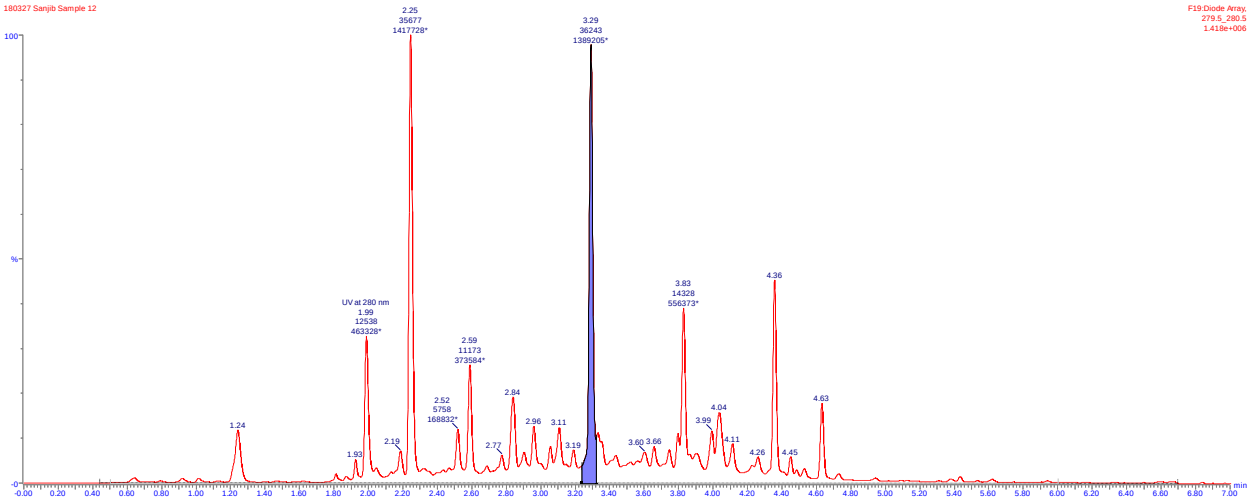

Fig. S37: UHPLC quantification of Oligomeric ETs by Diode-array detector at λ= 280 nm.

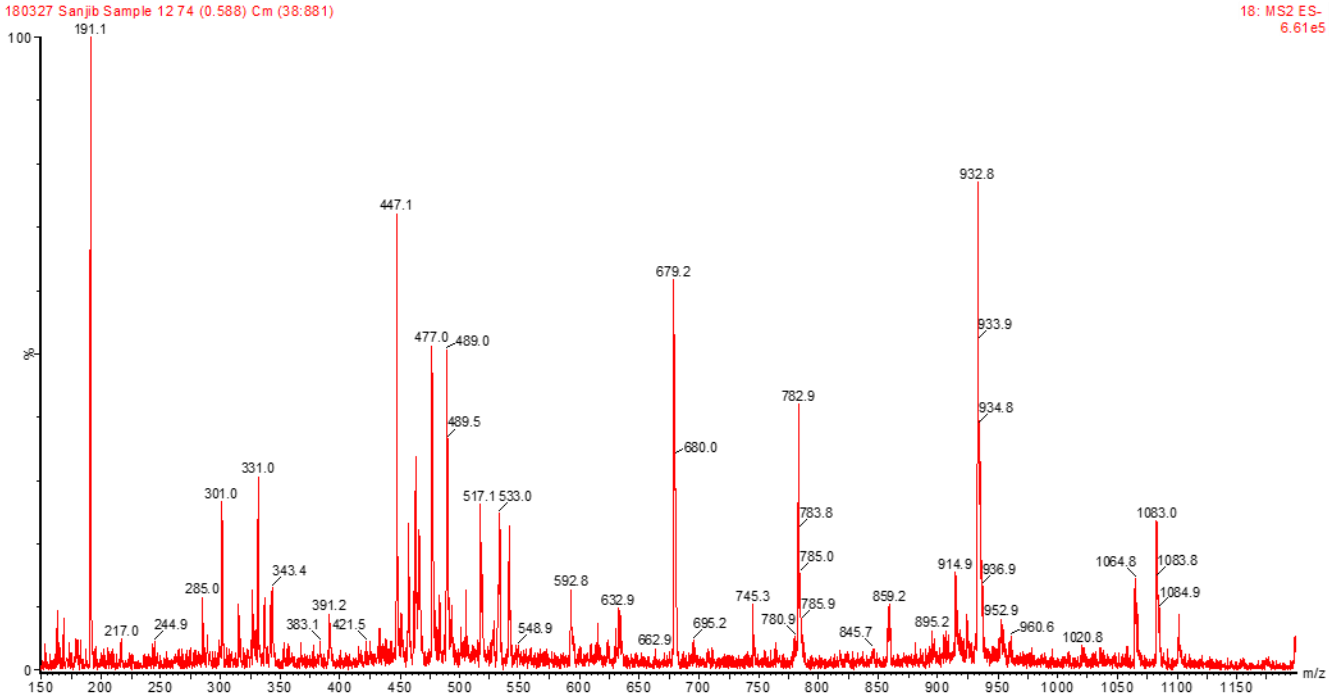

Fig. S38: MS spectrum from *m/z* 150 to 1200. ESI-MS identification: **Vescalagin** *m/z* at 933 $[M–H]^-$, *m/z* at 915 $[M–H_2O–H]^-$, *m/z* at 466 $[M–2H]^{2-}$, *m/z* at 457 $[M–H_2O–2H]^{2-}$, *m/z* at 301 [ellagic acid–H]$^-$; **Castalagin** *m/z* at 933 $[M–H]^-$, *m/z* at 466 $[M–2H]^{2-}$, *m/z* at 301 [ellagic acid–H]$^-$.[2]

Sample 12 (*Lythrum salicaria* L.)

Phenolic content	428716
Oligomeric ET	140203
Monomeric HT	73183
% of oligomeric ETs	32.70
% of monomeric HTs	17.07
% of HTs	49.77

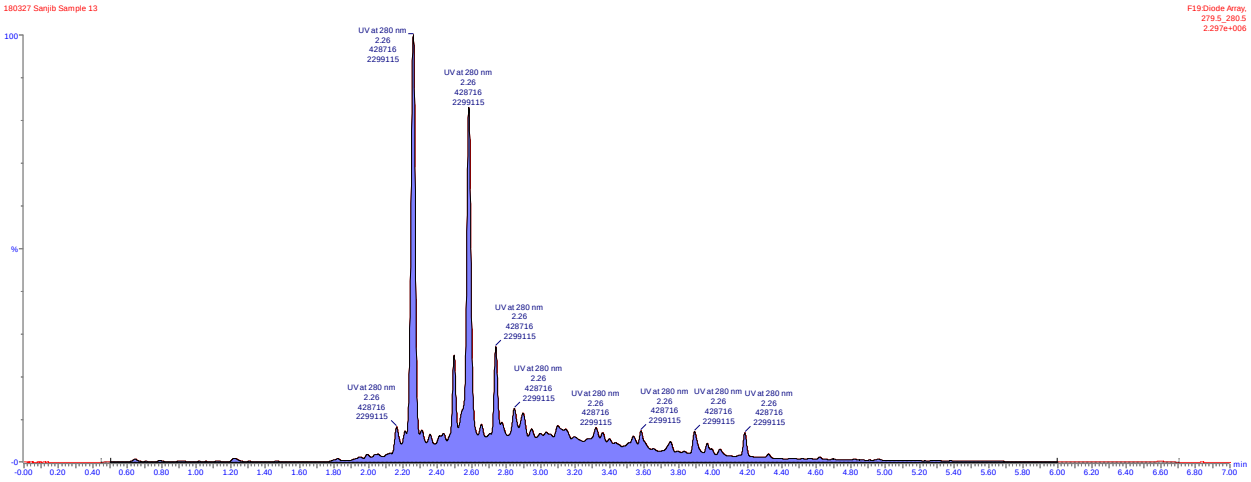

Fig. S39: UHPLC quantification of total phenolic content by Diode-array detector at λ= 280 nm.

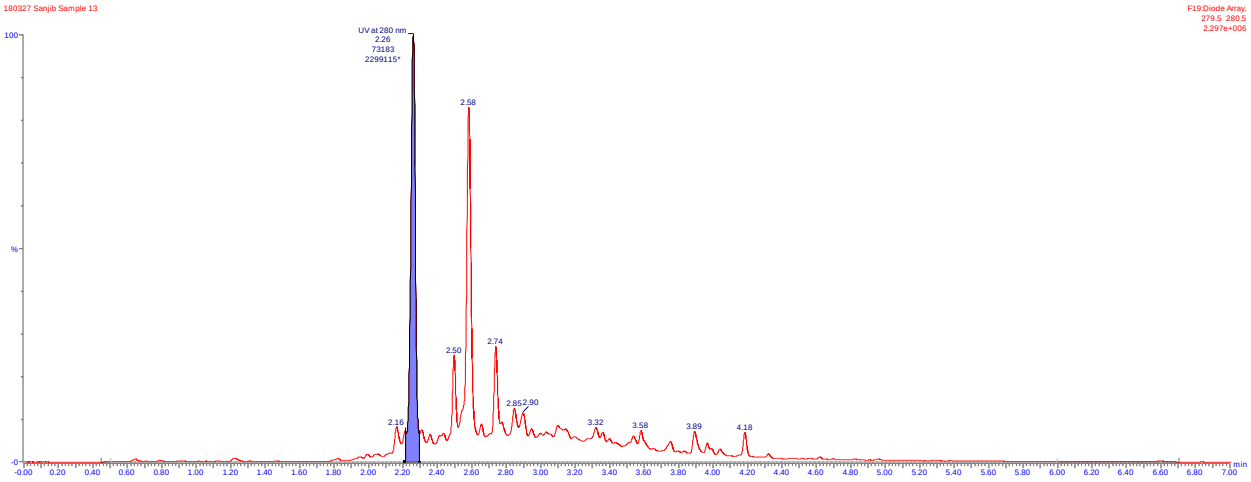

Fig. S40: UHPLC quantification of Monomeric HTs by Diode-array detector at λ= 280 nm.

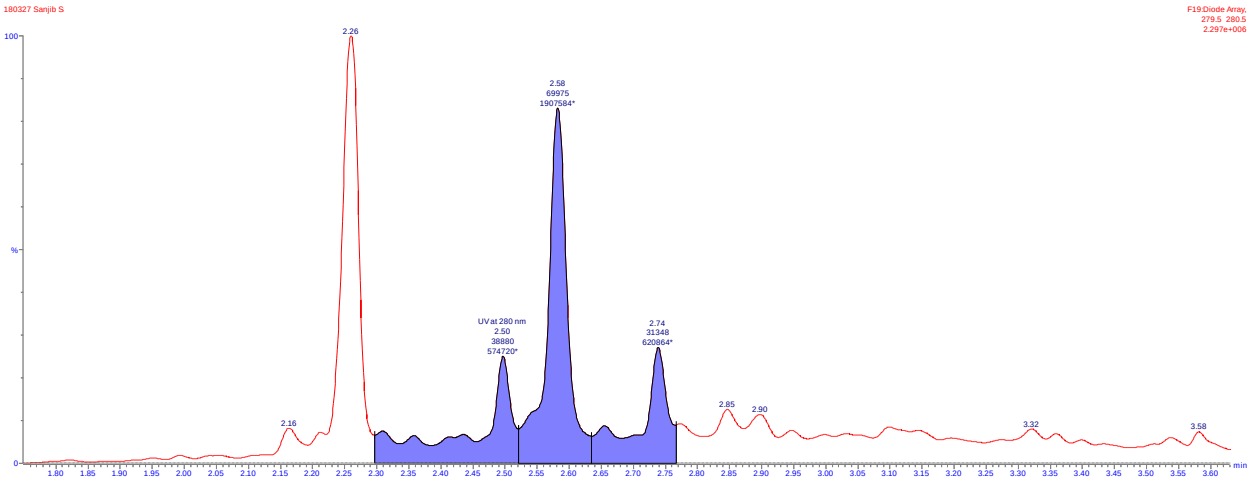

Fig. S41: UHPLC quantification of Oligomeric ETs by Diode-array detector at λ= 280 nm.

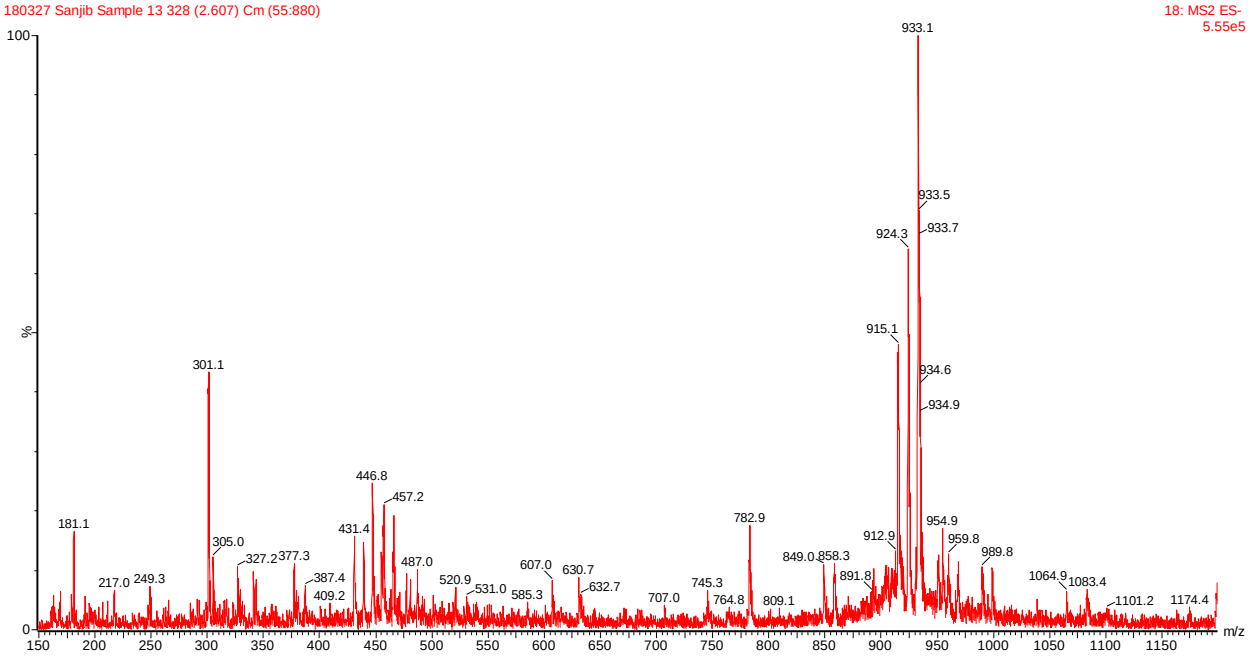

Fig. S42: MS spectrum from *m/z* 150 to 1200. ESI-MS identification: **Salicarinin A** *m/z* at 933 $[M–2H]^{2–}$; **Salicarinin B** *m/z* at 933 $[M–2H]^{2–}$.[9]