

## Supporting Information

### UPLC-ESI-QTOF-MS/MS Profiling, Antioxidant, and Cytotoxicity Potentials of *Marrubium vulgare* L. Extracts: Experimental Analysis and Computational Validation

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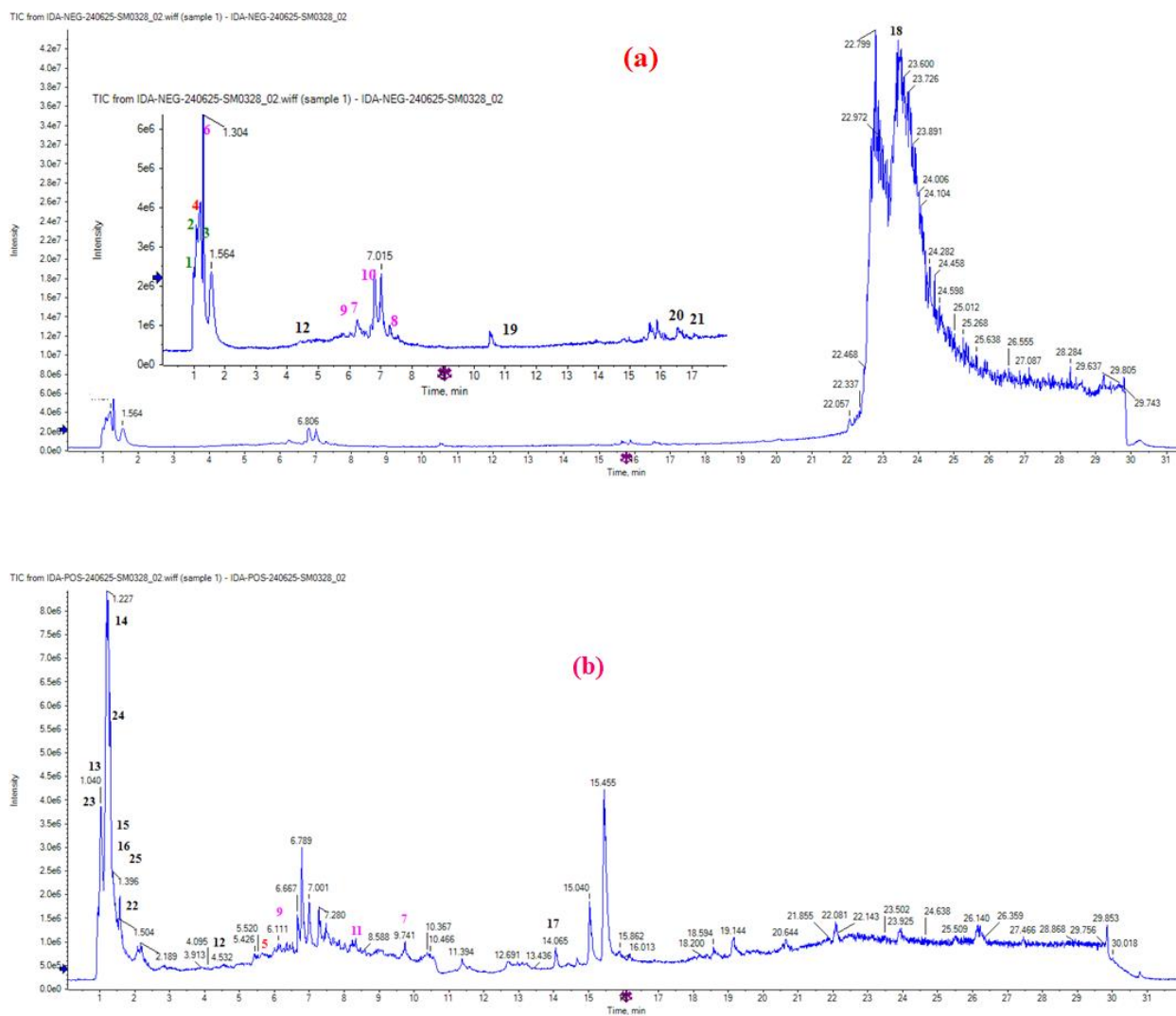
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**Abstract:** Plant extracts are emerging as valuable options for food additives and therapeutic treatments. This study evaluated the phytochemical profile, antioxidant activity, and cytotoxicity of aerial parts of *Marrubium vulgare* L. crude extract (MVCE) and its subfractions. The MVCE (80% ethanol) contained steroids, phenolic compounds, flavonoids, terpenes, and cardiac glycosides, with total phenolic content (TPC) and total flavonoid content (TFC) of  $14.96 \pm 0.12$  mg GAE/g DW and  $12.27 \pm 0.63$  mg RE/g DW, respectively. All MV extracts exhibited potent antioxidant activity against DPPH<sup>•</sup> (0.106–1.864 mg/mL) and ABTS<sup>•+</sup> (0.298–17.084 mg/mL). The MV residual aqueous fraction (MVRF) showed significant cytotoxicity against human cancer cell lines, including MCF7 (IC<sub>50</sub> =  $5.47 \pm 1.32$  µg/mL), HT29 (IC<sub>50</sub> =  $17.48 \pm 1.47$  µg/mL), and SW480 (IC<sub>50</sub> =  $7.51 \pm 0.36$  µg/mL). Ultra-performance liquid chromatography-mass spectrometry identified 26 bioactive compounds, with malic acid, caffeic acid, chlorogenic acid, kaempferol-3-glucuronide, and L-tryptophan as the major ones. Molecular docking revealed strong binding affinities of the above compounds to breast (PDB ID: 6CHZ) and colorectal cancer (PDB ID: 1HVY) proteins. Pharmacokinetic and toxicological studies confirmed their safety and efficacy, supporting MVRF as a potential therapeutic agent. These findings highlight MV as a promising candidate for future anticancer research.

**Keywords:** *Marrubium vulgare* L., Antioxidant, Cytotoxicity, UPLC-ESI-QTOF-MS/MS, In silico study

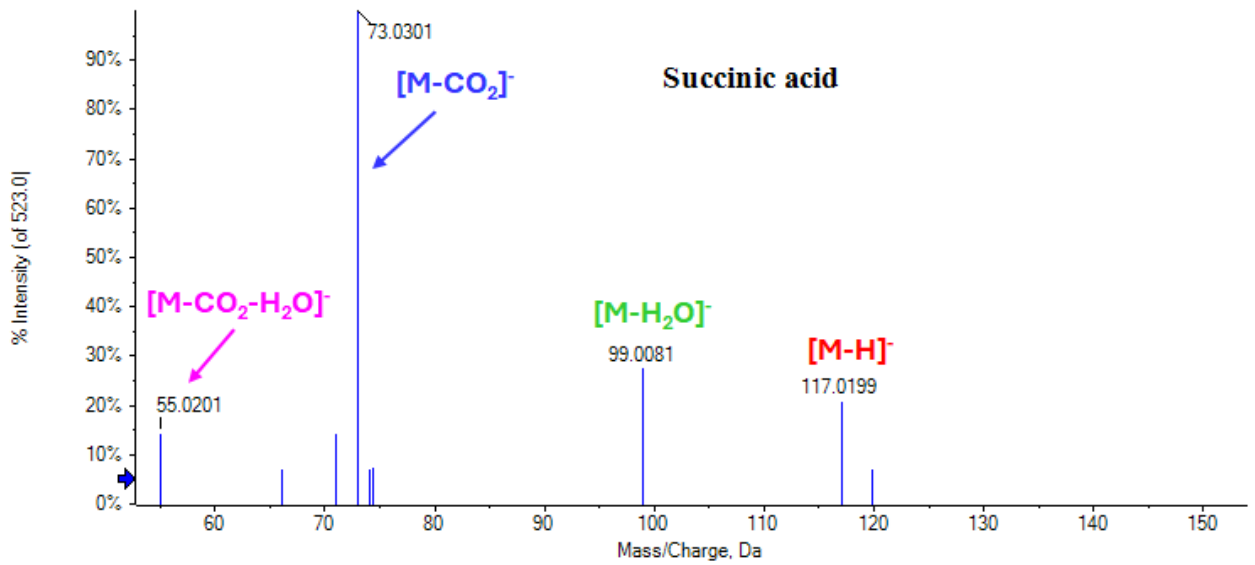
## Supporting Results

### Supporting Information Figures S1-S5

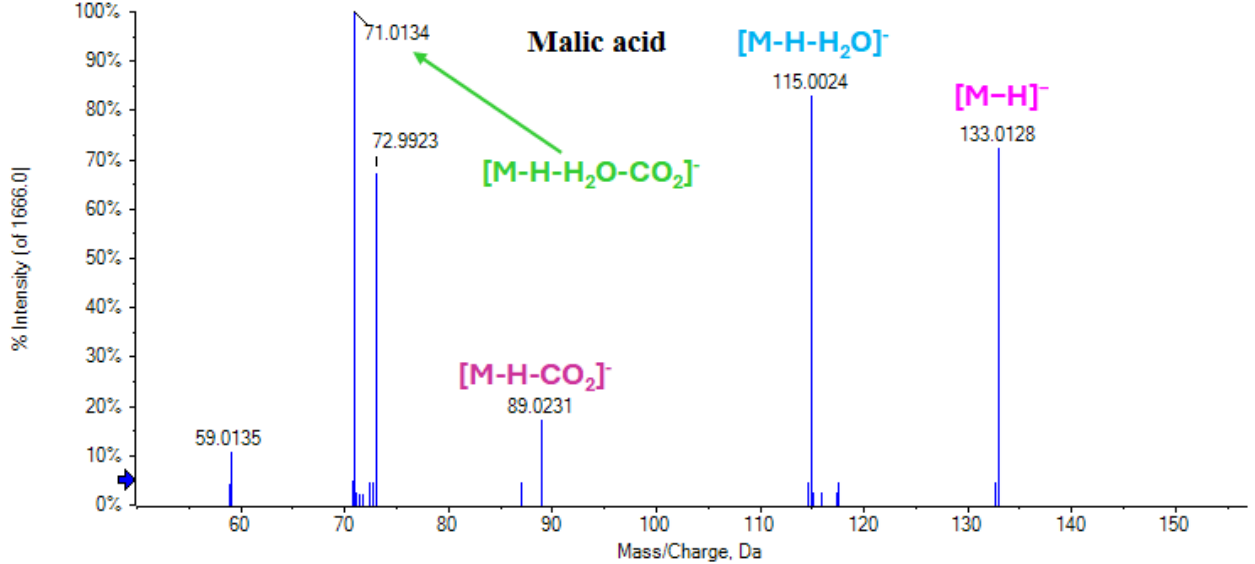


**Fig. S1.** Total ion chromatogram (TIC) of MVRF in (a) ESI<sup>-</sup> mode, (b) ESI<sup>+</sup> mode

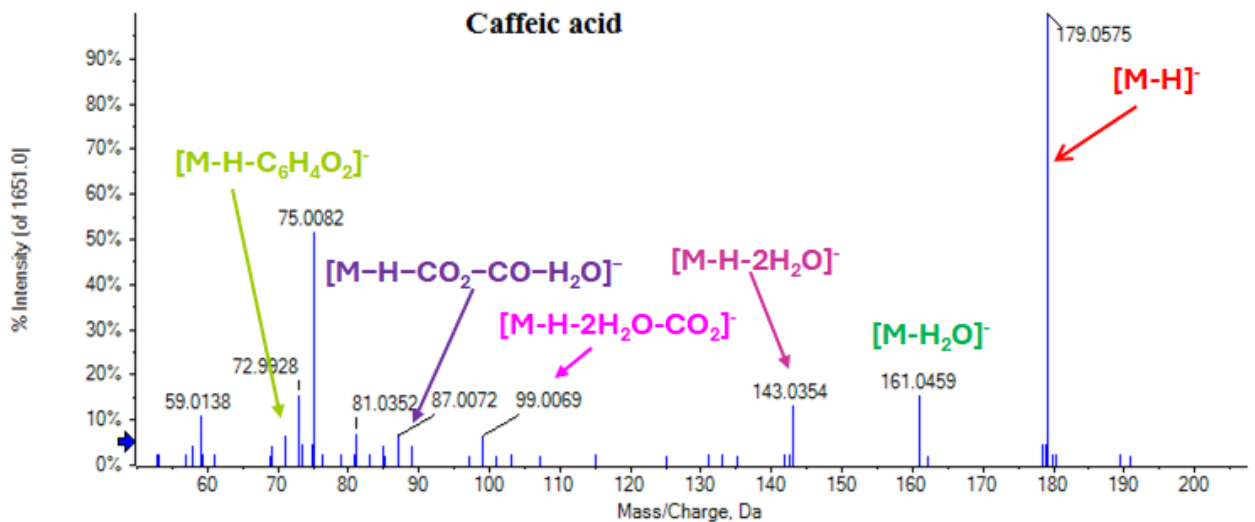
Spectrum from IDA-NEG-240625-SM0328\_02.wiff (sample 1) - IDA-NEG-...625-SM0328\_02, Experiment 3, -TOF MS<sup>2</sup> (50 - 1000) from 1.008 min  
Precursor: 117.0 Da



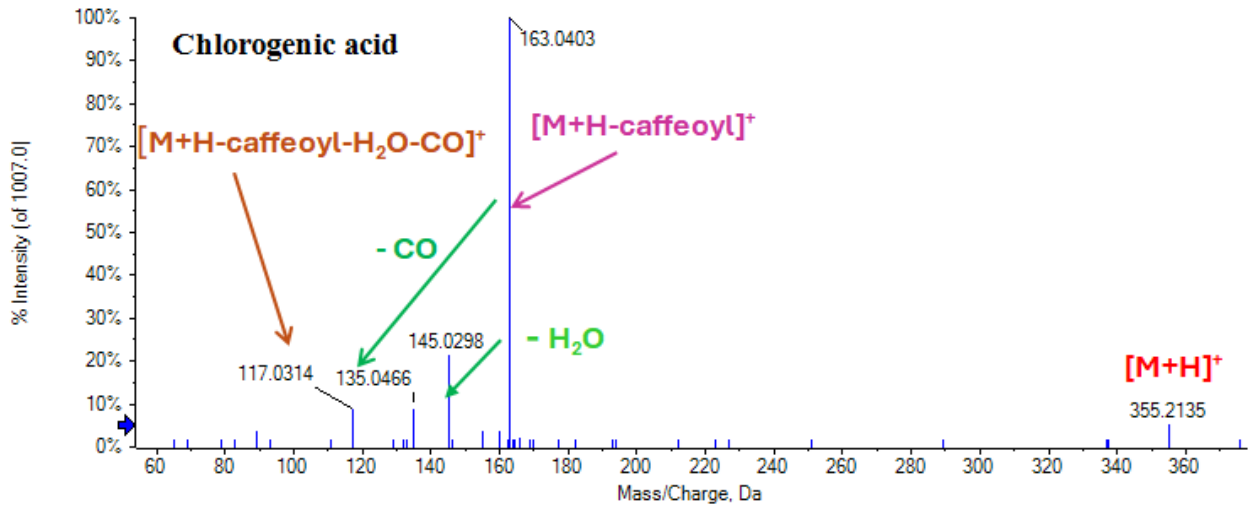
Spectrum from IDA-NEG-240625-SM0328\_02.wiff (sample 1) - IDA-NEG-...625-SM0328\_02, Experiment 2, -TOF MS<sup>2</sup> (50 - 1000) from 1.046 min  
Precursor: 133.0 Da



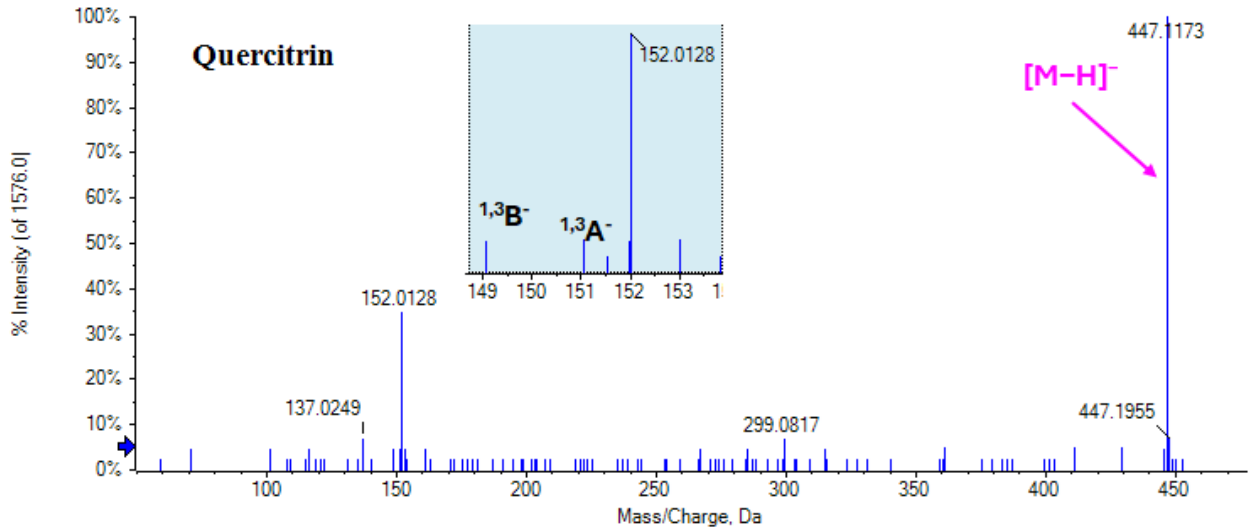
Spectrum from IDA-NEG-240625-SM0328\_02.wiff (sample 1) - IDA-NEG-...625-SM0328\_02, Experiment 7, -TOF MS<sup>2</sup> (50 - 1000) from 1.087 min  
Precursor: 179.1 Da



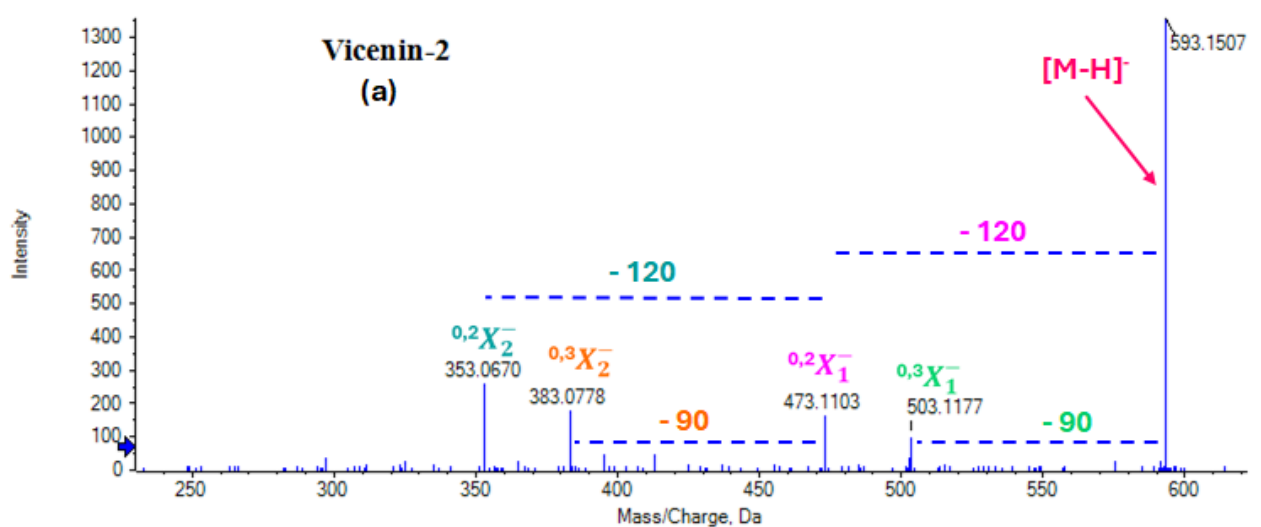
Spectrum from IDA-POS-240625-SM0328\_02.wiff (sample 1) - IDA-POS-...25-SM0328\_02, Experiment 2, +TOF MS<sup>2</sup> (50 - 1000) from 6.087 min  
 Precursor: 355.1 Da, CE: 35.0



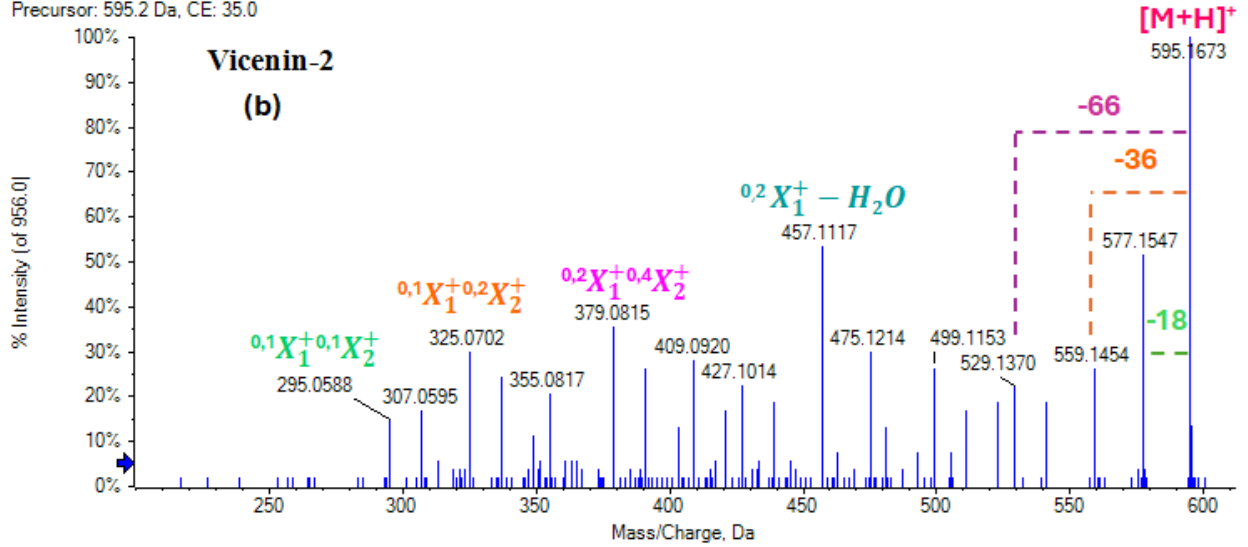
Spectrum from IDA-NEG-240625-SM0328\_02.wiff (sample 1) - IDA-NEG-...25-SM0328\_02, Experiment 13, -TOF MS<sup>2</sup> (50 - 1000) from 1.309 min  
 Precursor: 447.1 Da



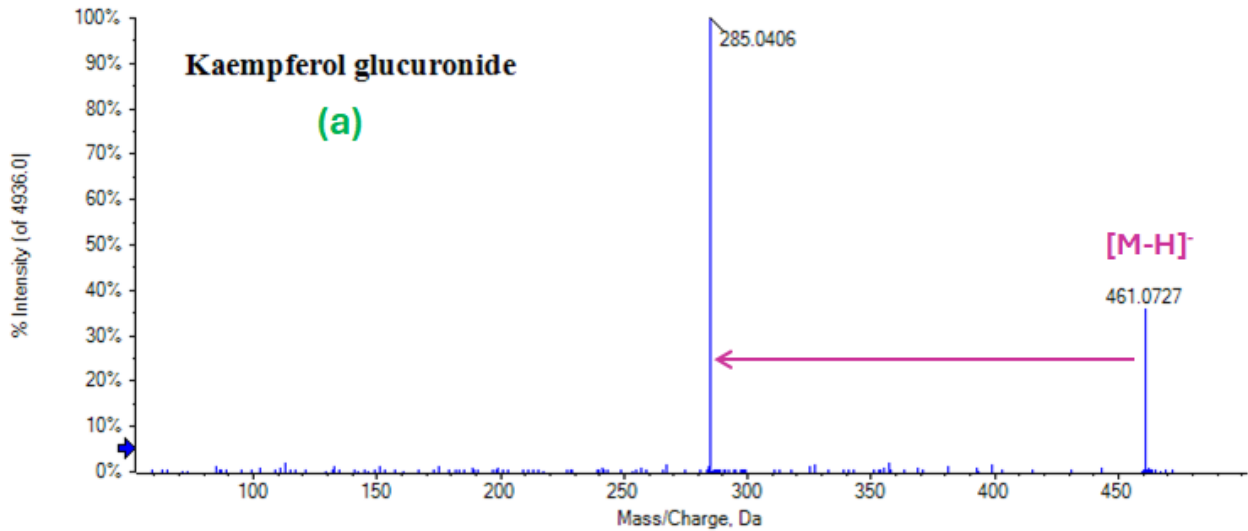
Spectrum from IDA-NEG-240625-SM0328\_02.wiff (sample 1) - IDA-NEG-...625-SM0328\_02, Experiment 3, -TOF MS<sup>2</sup> (50 - 1000) from 5.785 min  
 Precursor: 593.2 Da



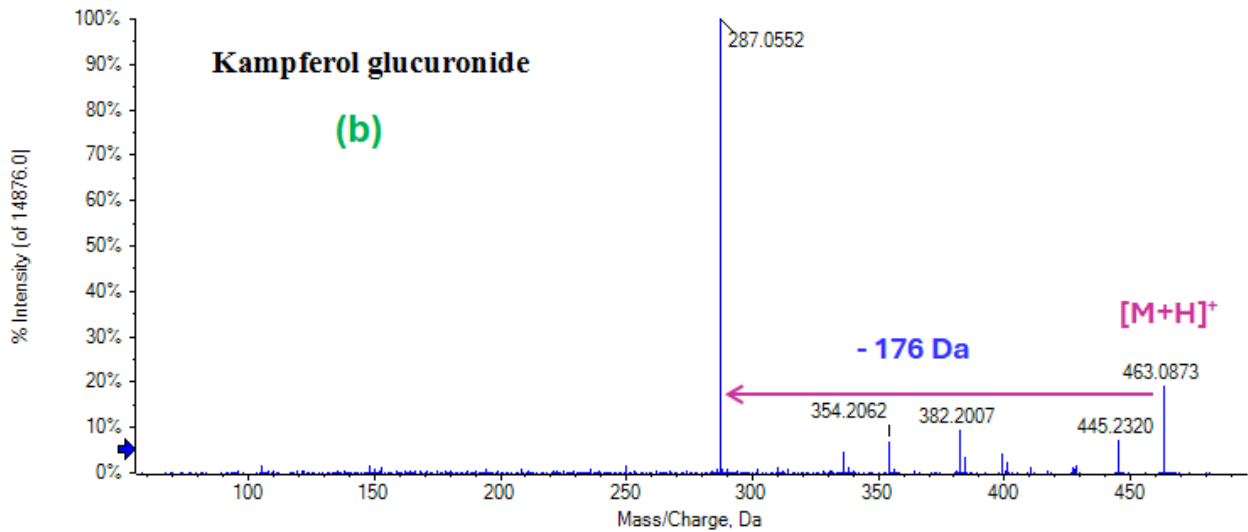
Spectrum from IDA-POS-240625-SM0328\_02.wiff (sample 1) - IDA-POS-...25-SM0328\_02, Experiment 6, +TOF MS<sup>2</sup> (50 - 1000) from 6.117 min  
 Precursor: 595.2 Da, CE: 35.0



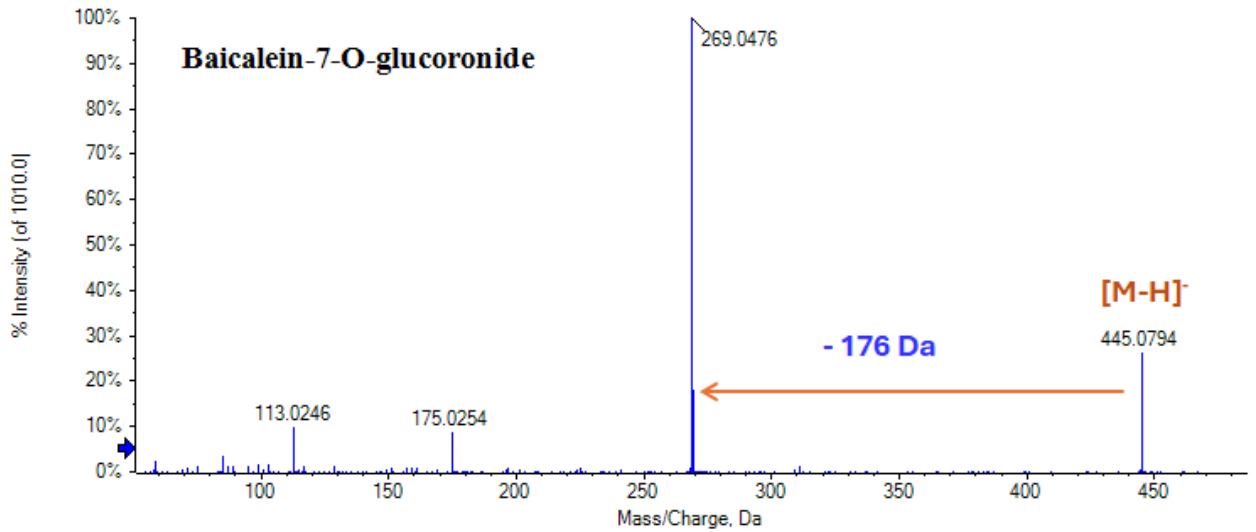
Spectrum from IDA-NEG-240625-SM0328\_02.wiff (sample 1) - IDA-NEG-...625-SM0328\_02, Experiment 5, -TOF MS<sup>2</sup> (50 - 1000) from 6.251 min  
 Precursor: 461.1 Da



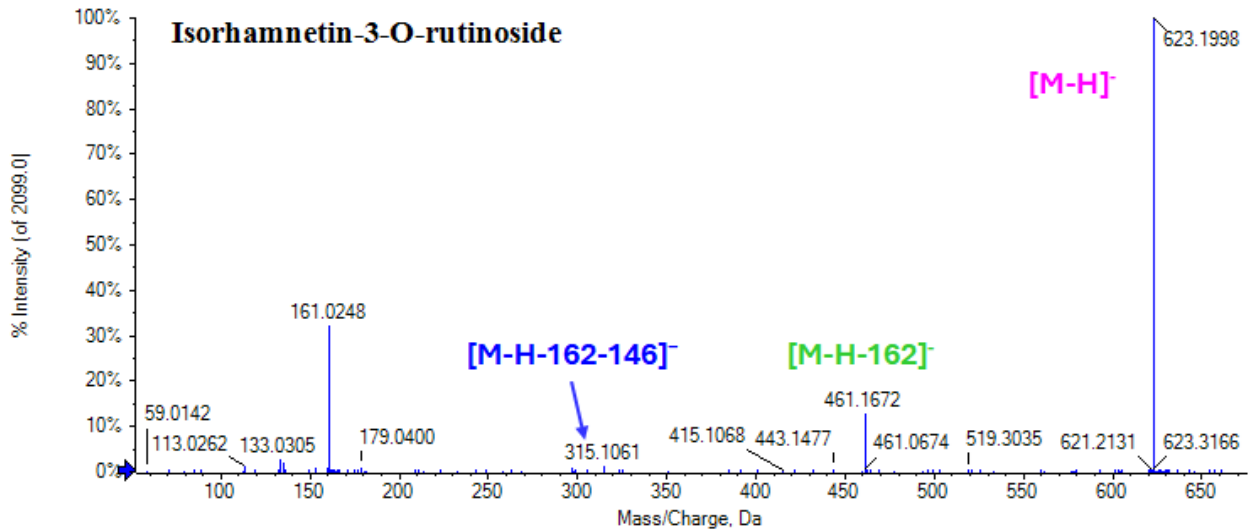
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 Precursor: 463.2 Da, CE: 35.0



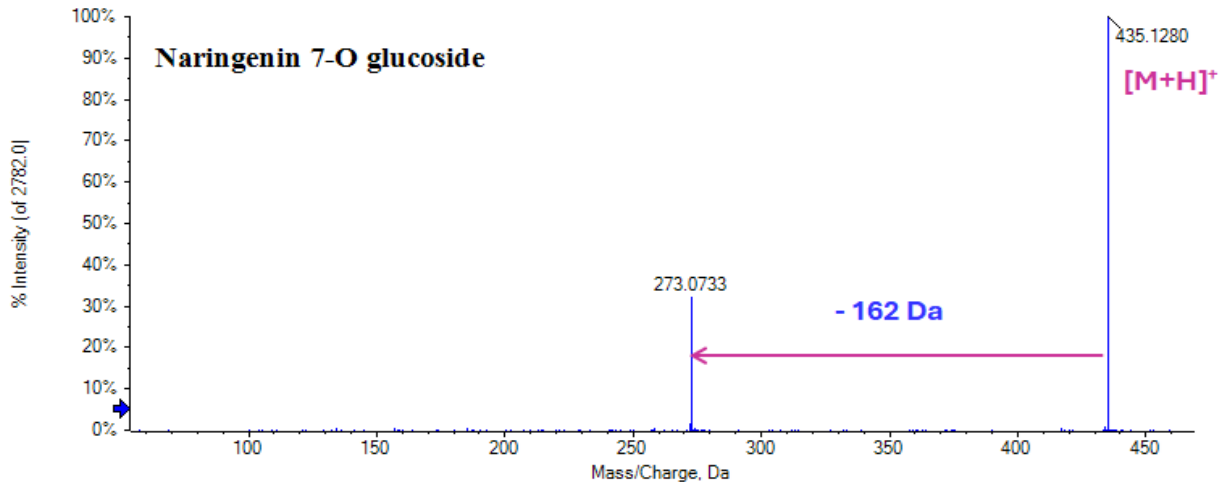
Spectrum from IDA-NEG-240625-SM0328\_02.wiff (sample 1) - IDA-NEG-...625-SM0328\_02, Experiment 2, -TOF MS<sup>2</sup> (50 - 1000) from 6.732 min  
Precursor: 445.1 Da



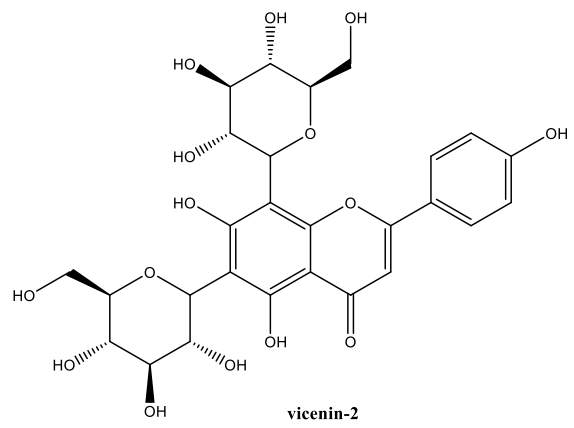
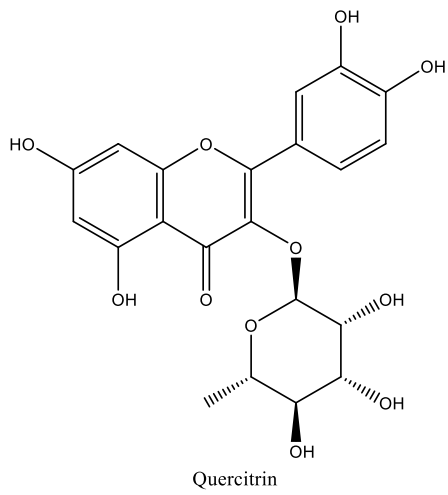
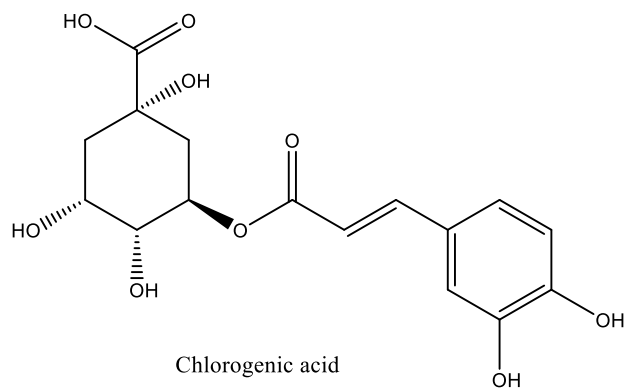
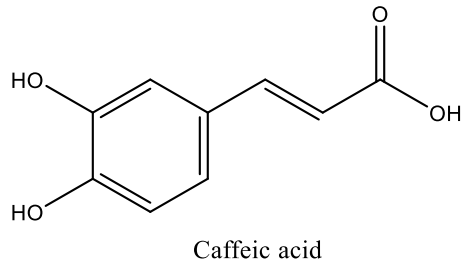
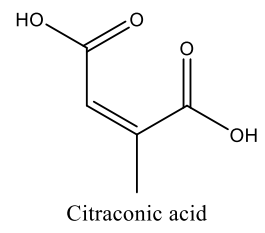
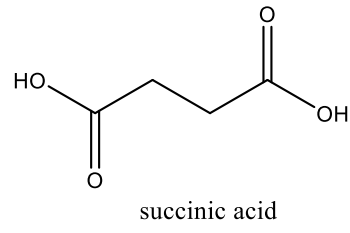
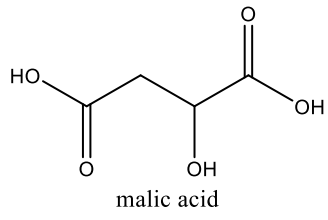
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Precursor: 623.2 Da

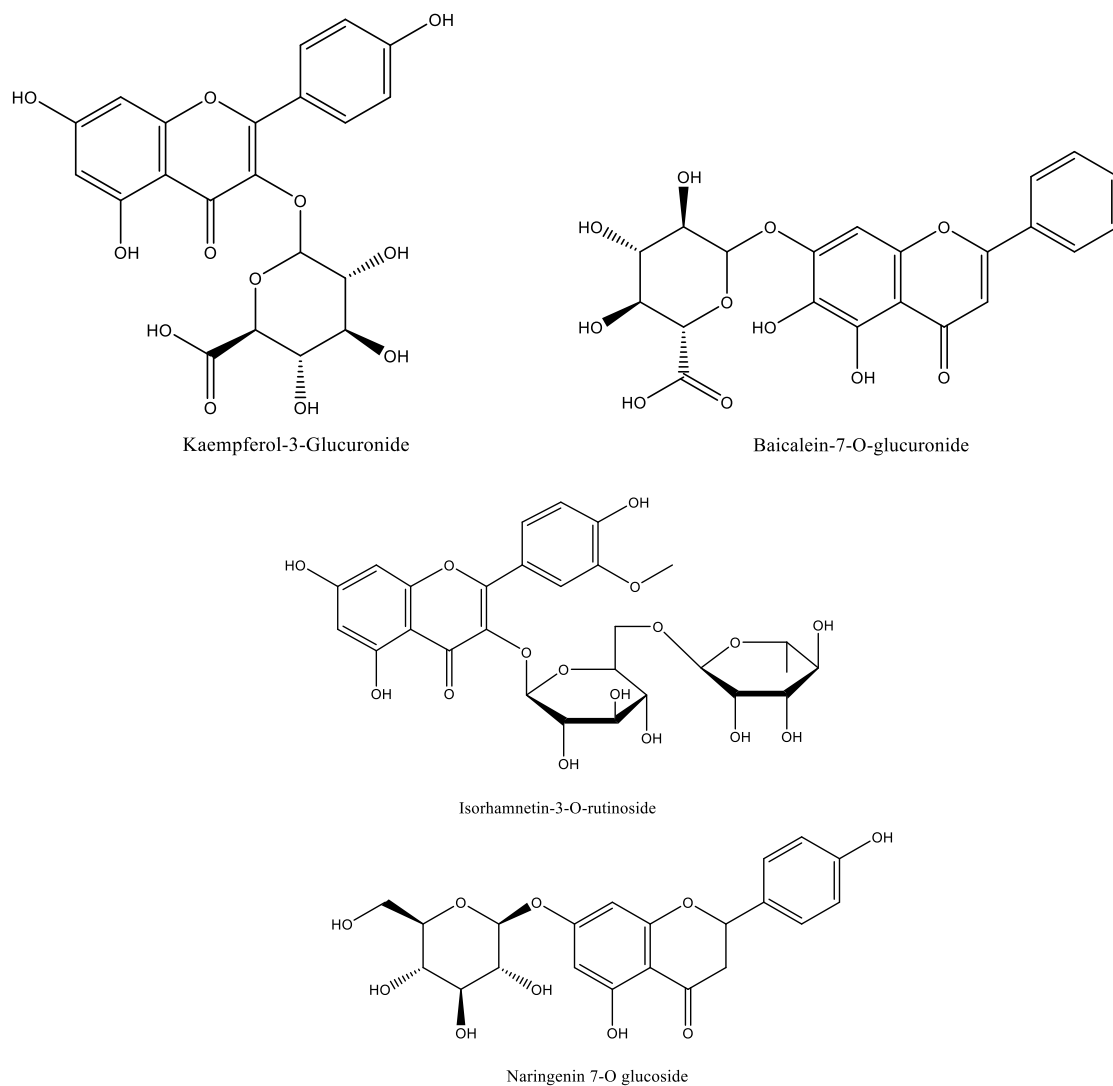


Spectrum from IDA-POS-240625-SM0328\_02.wiff (sample 1) - IDA-POS-...25-SM0328\_02, Experiment 2, +TOF MS<sup>2</sup> (50 - 1000) from 8.845 min  
Precursor: 435.1 Da, CE: 35.0

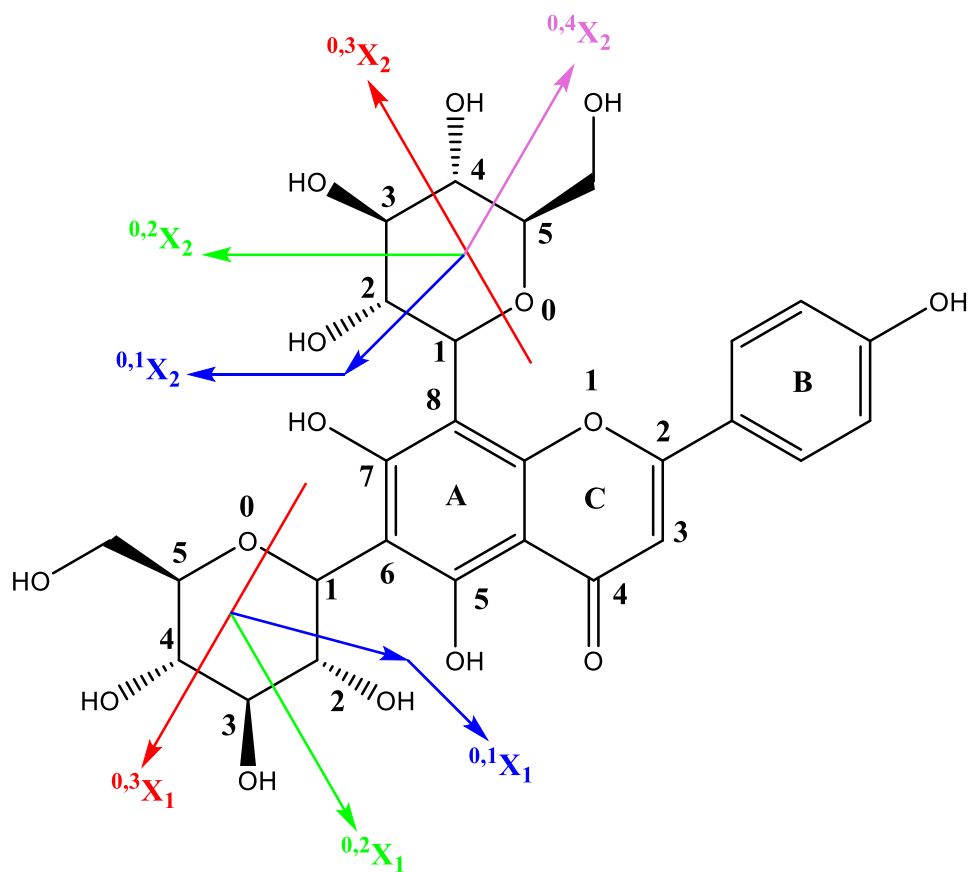


**Fig. S2.** MS/MS spectra and fragmentation patterns of some identified secondary metabolites in MVRF





**Fig. S3.** Structure of identified phenolic compounds in RFMV by UPLC-ESI-QTOF-MS/MS



**Fig. S4.** Fragmentation sites of vicenin-2 at C-glycosidic hexose moieties