

Supplementary Information

Hempseed Lignanamides Rich-Fraction: Chemical Investigation and Cytotoxicity towards U-87 Glioblastoma Cells

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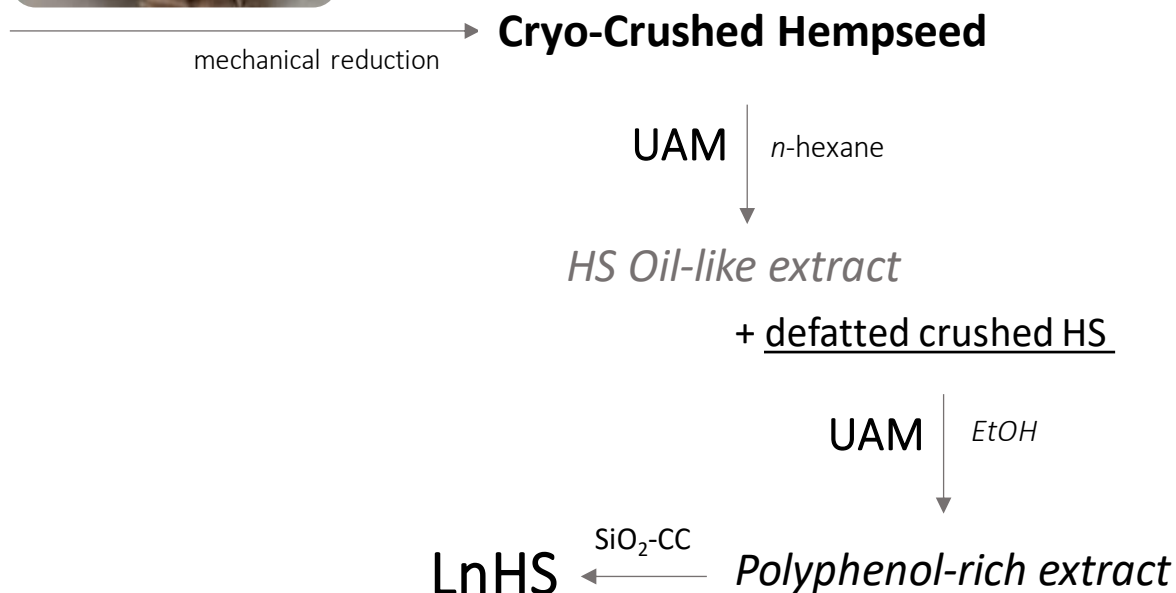


Figure 1. Simplified extraction and fractionation scheme of cryo-crushed hempseeds.

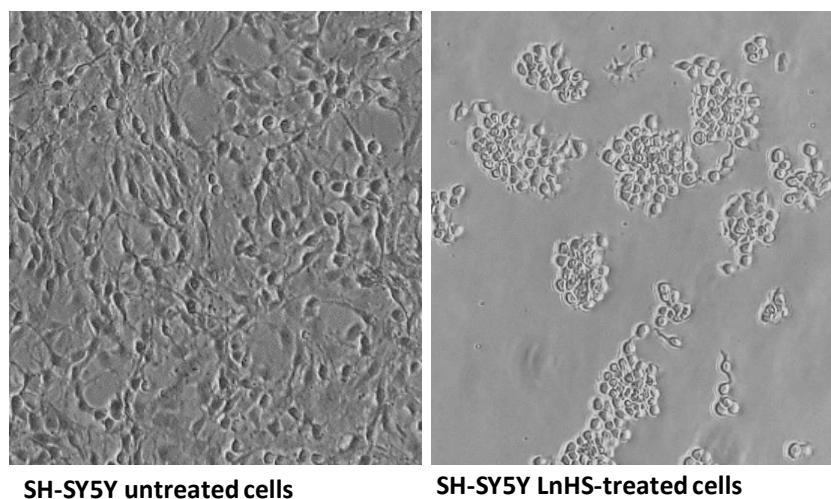


Figure 2. Morphological changes in SH-SY5Y cells treated with LnHS fraction in respect to untreated cells. Representative images were acquired by Inverted Phase Contrast Brightfield Zeiss Primo Vert Microscope.

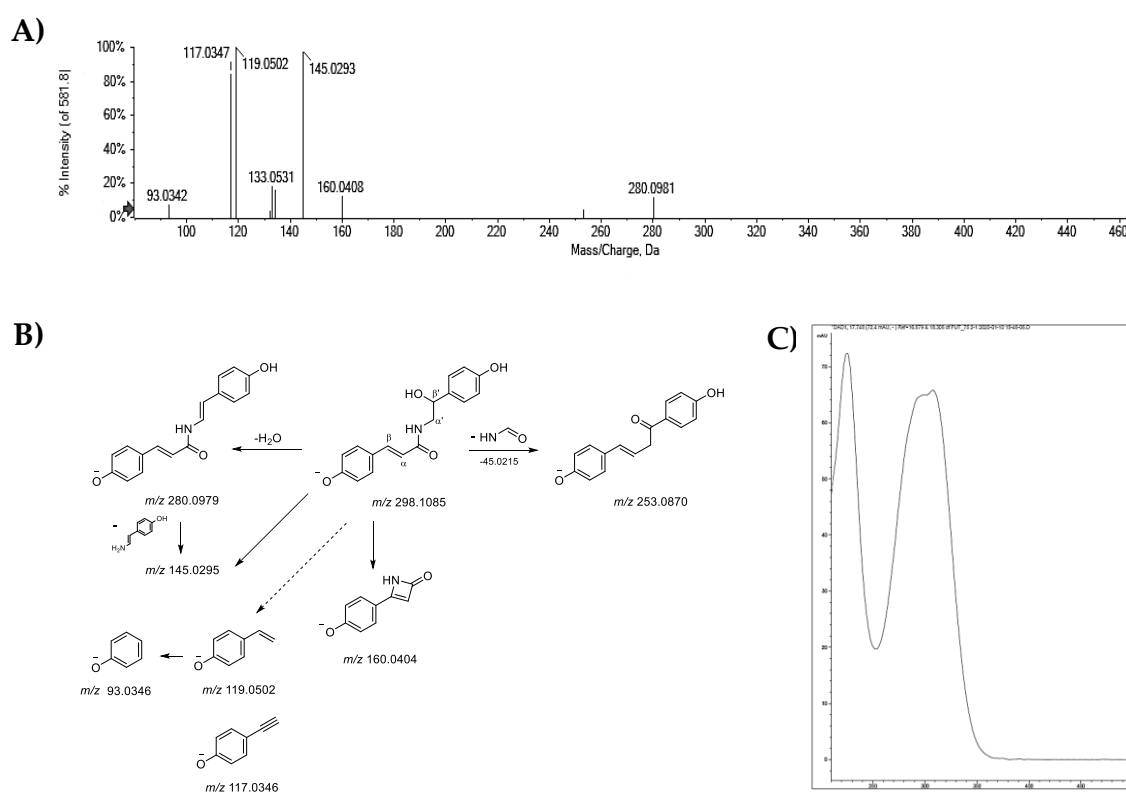


Figure 3. A) TOF-MS/MS spectrum; B) proposed fragmentation pathway of the $[M-H]^-$ ion; C) UV-DAD spectrum for compound **2**. In B panel, the theoretical m/z value is reported below each structure.

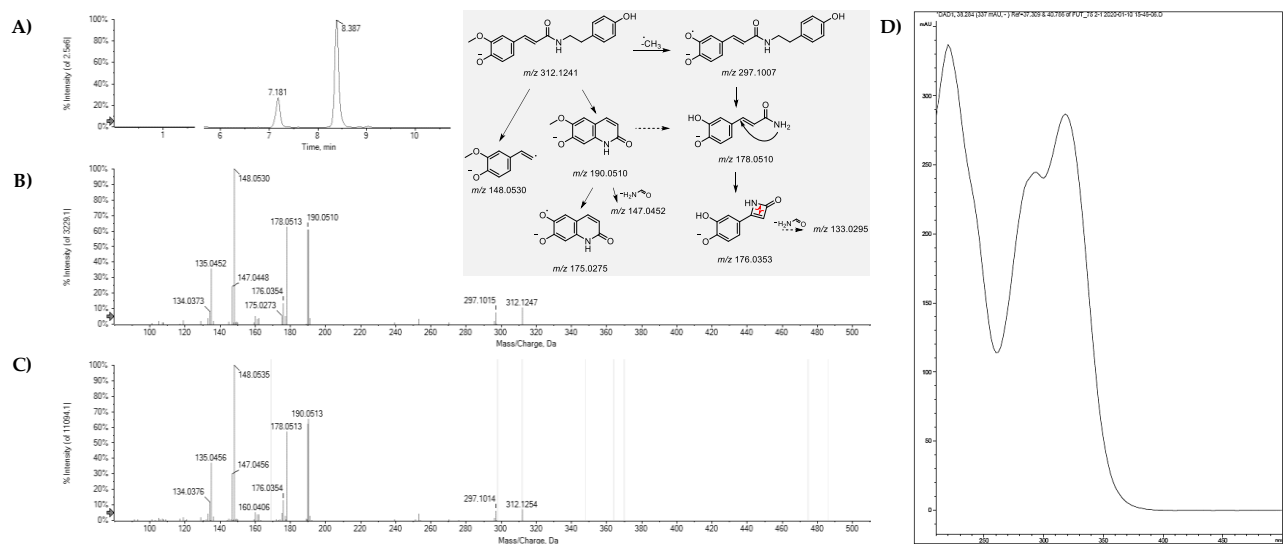


Figure 4. A) Extracted ion chromatogram (XIC) of the $[M-H]^-$ ion at m/z 312.124 ± 0.025 ; TOF-MS/MS spectra of B) compound **8**, and C) compound **17**. In the grey panel the tentatively proposed fragmentation pathway of the $[M-H]^-$ ions. Representative UV-DAD spectrum, acquired under peak **17**, is reported in panel D.

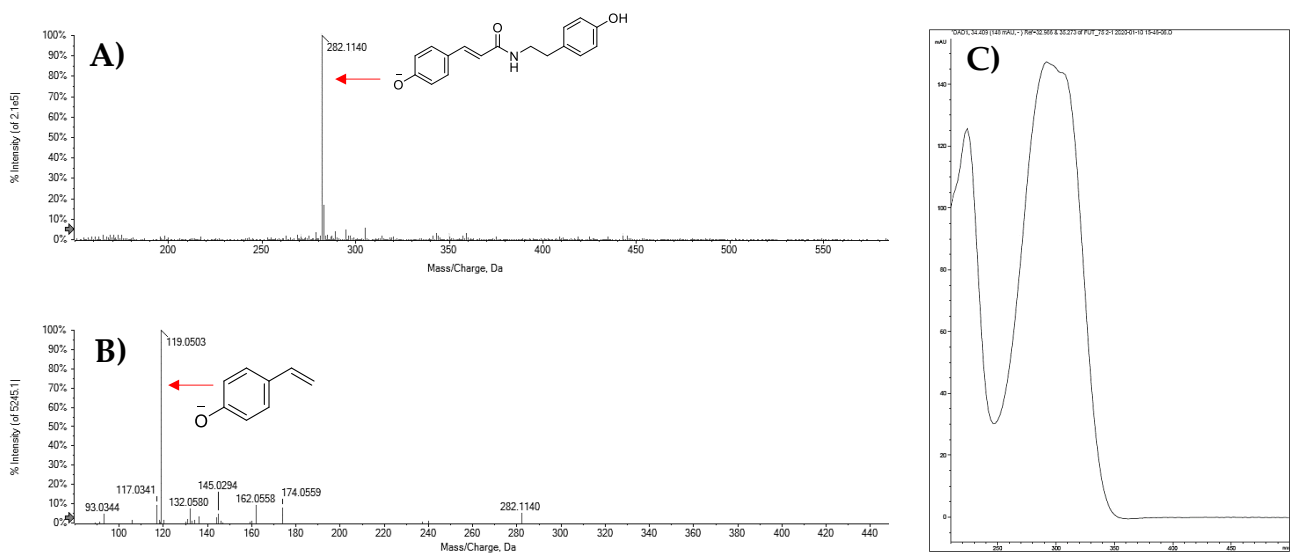


Figure 5. A) TOF-MS spectrum for compound **11**; B) TOF MS/MS spectrum of the $[M-H]^-$ ion; C) UV-DAD spectrum.

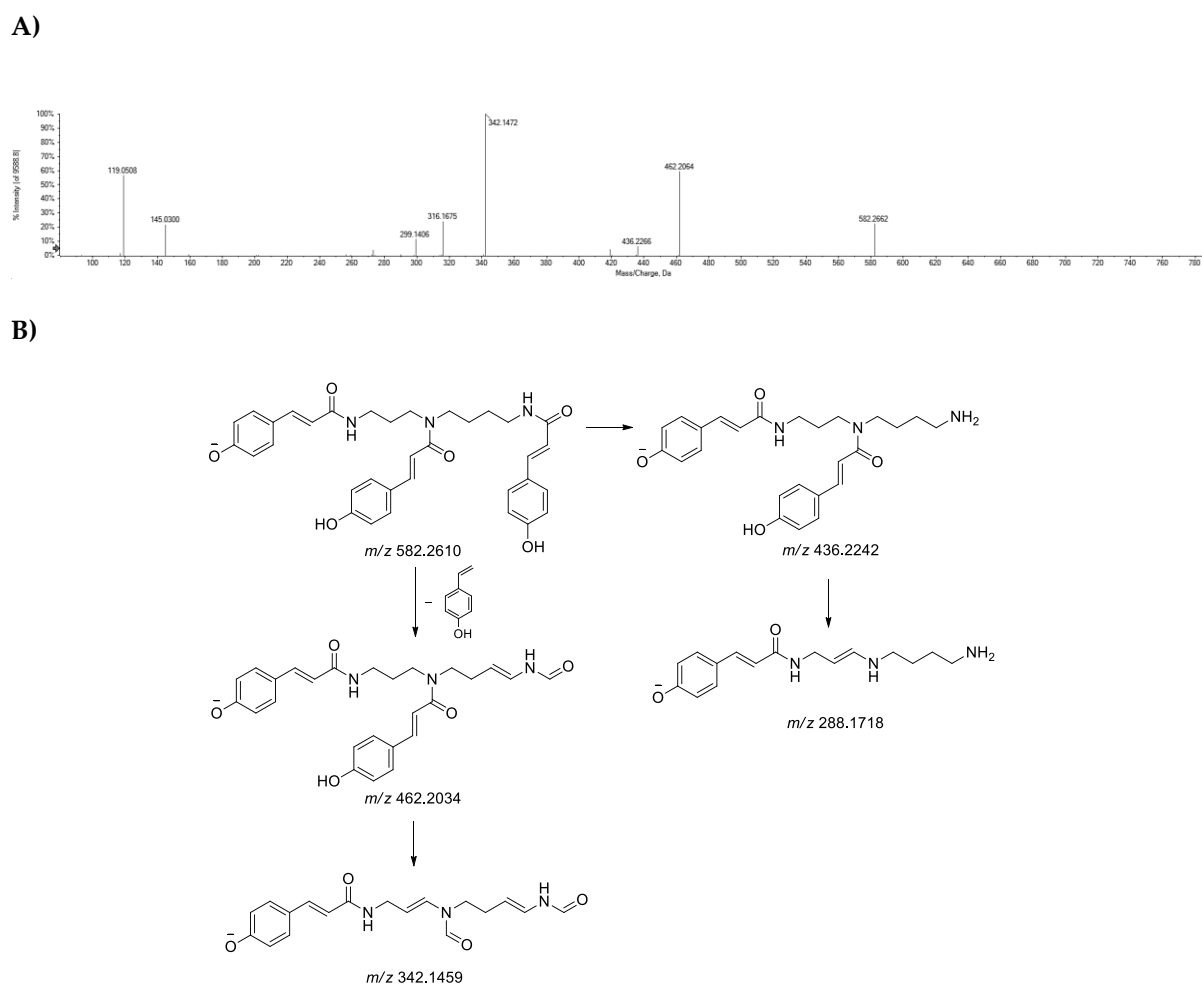


Figure 6. A) TOF-MS/MS spectrum and B) proposed fragmentation pathway of the $[M-H]^-$ ion for compound **33**; theoretical m/z values are reported below each structure.

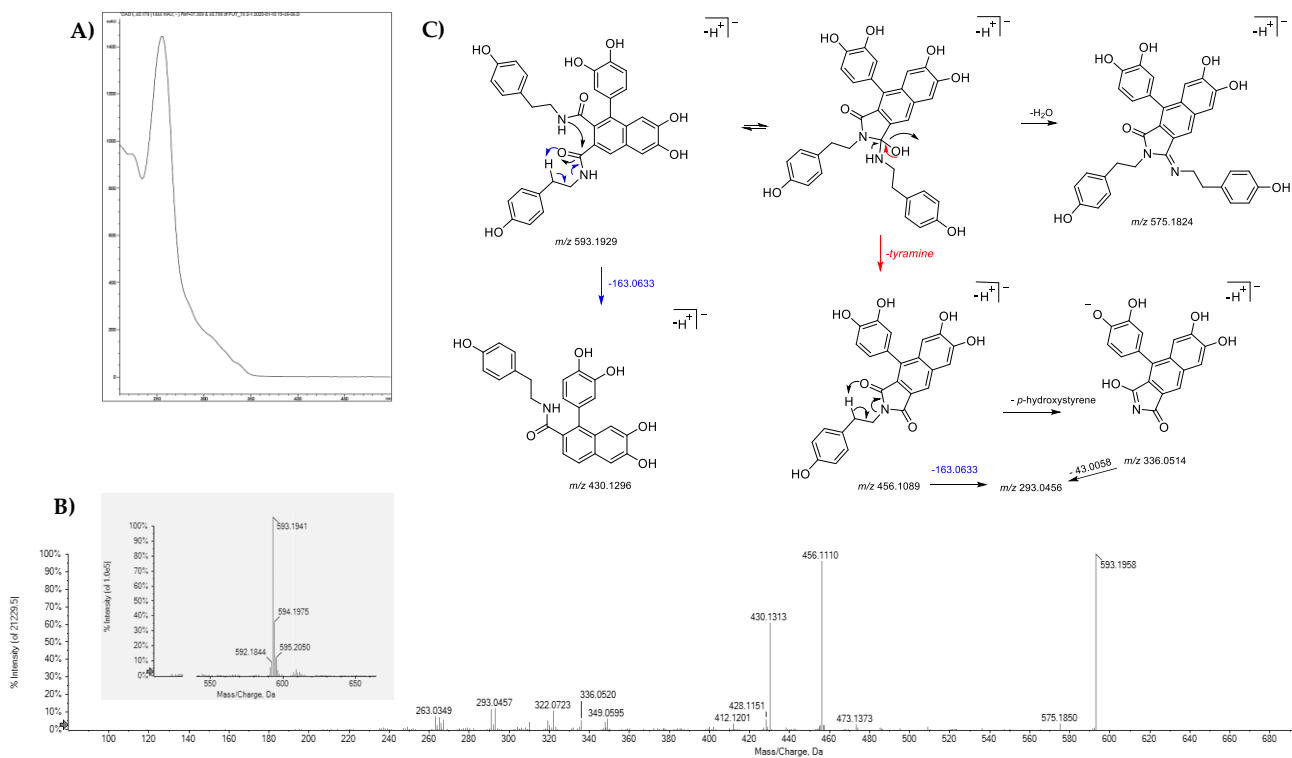


Figure 7. Compound **16** A) UV-DAD spectrum; B) TOF-MS/MS spectrum (in grey panel TOF-MS spectrum is reported). C) Proposed fragmentation pathway of the $[M-H]^-$ ion (theoretical m/z values are reported below each structure).

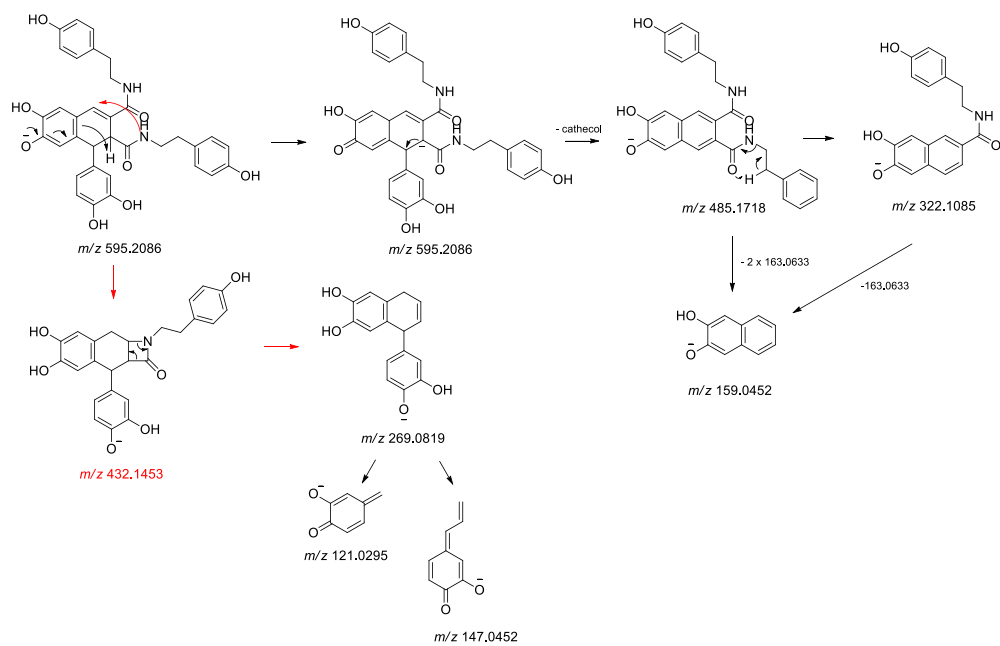
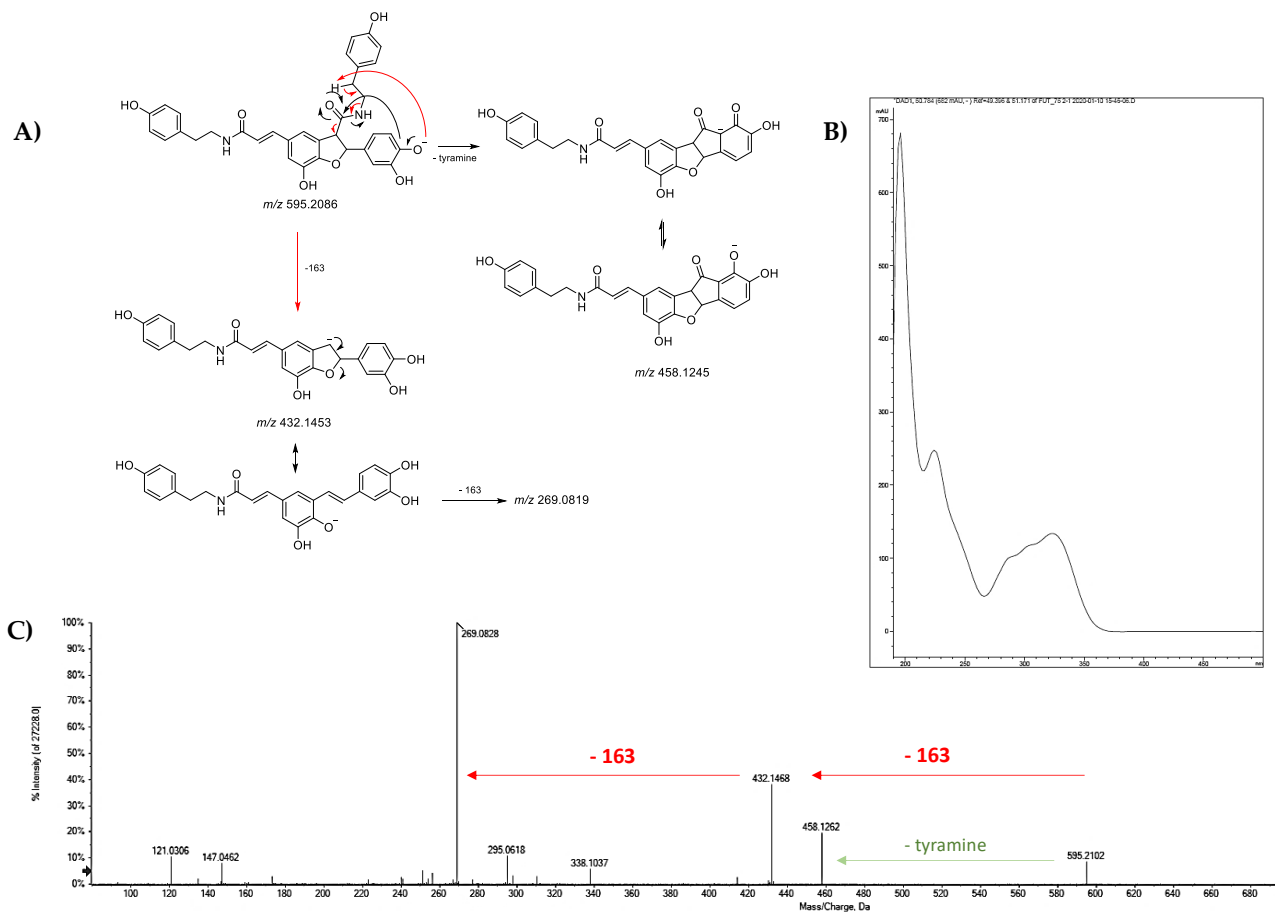


Figure 8. Proposed fragmentation pathway of the $[M-H]^-$ ion relative to cannabisin B isomers (theoretical m/z values are reported below each structure).



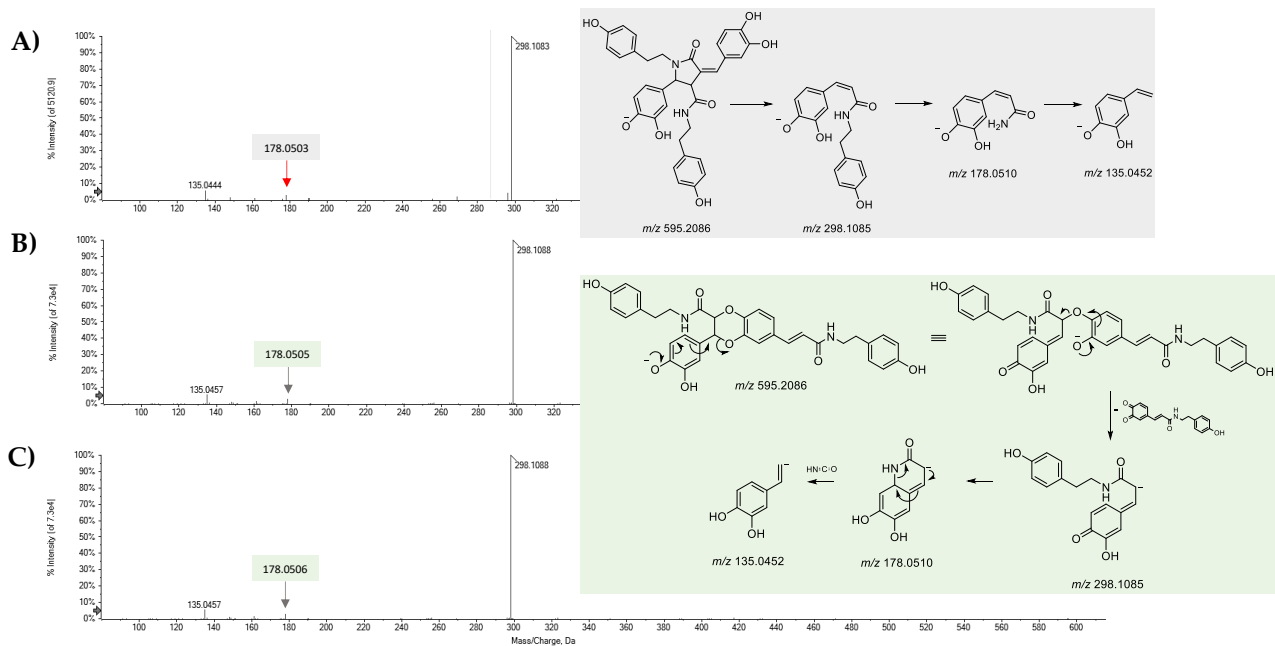


Figure 10. TOF-MS/MS spectra of compounds **32**, **36** and **38** (A, B, and C, respectively). Proposed fragmentation pathway of the $[M-H]^-$ ion of the compound **32** is reported in grey panel, whereas that of **36** and **38** is in light green panel (theoretical m/z values are reported below each structure).

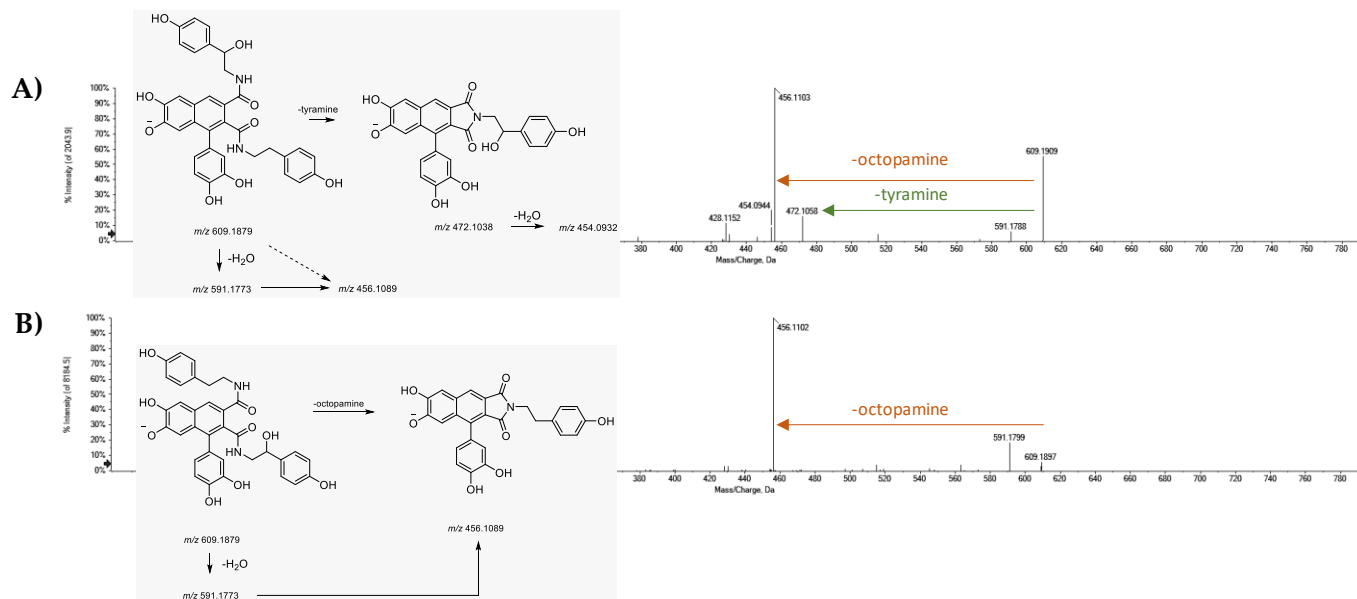
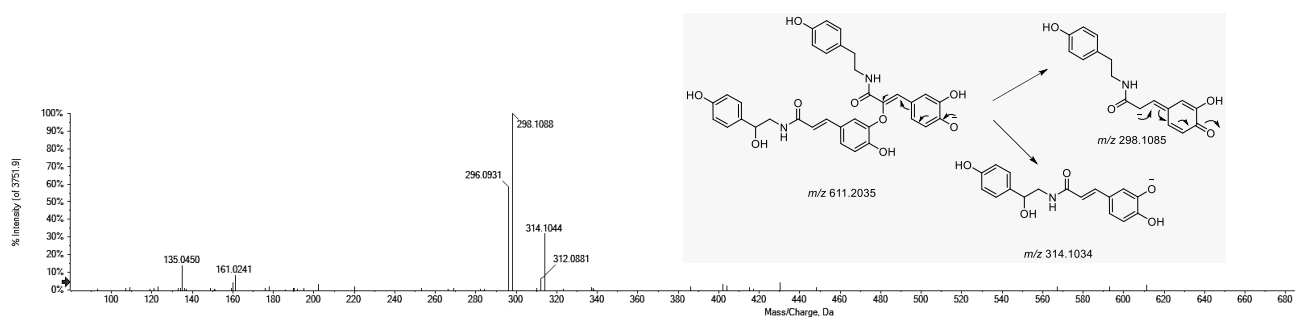
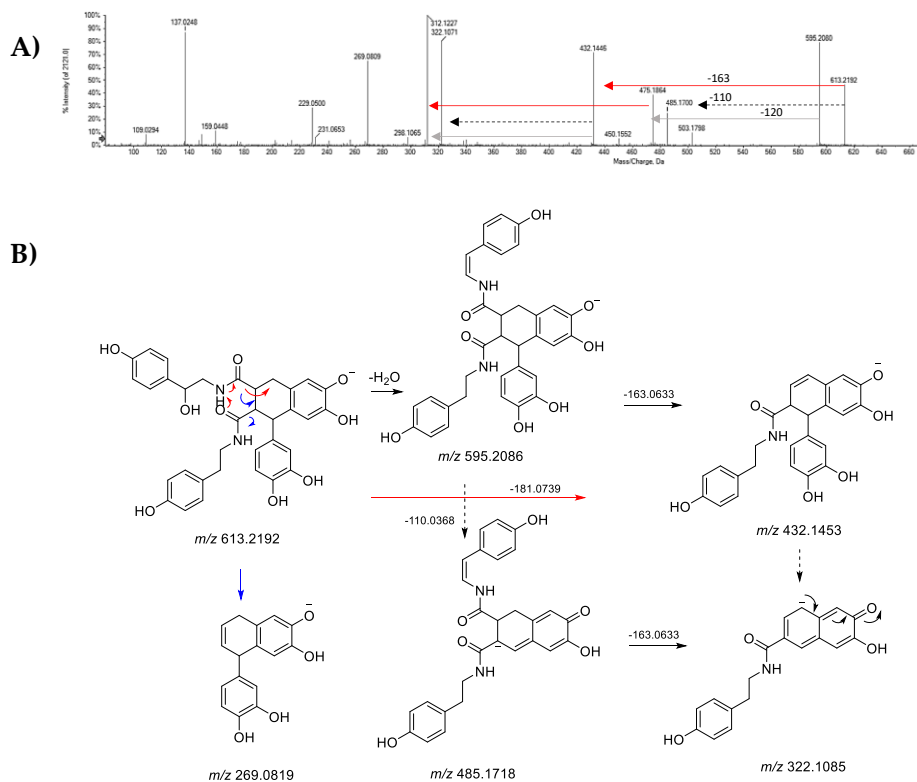


Figure 11. TOF-MS/MS spectra of compounds 4 and 6 (A, and B, respectively). Proposed fragmentation pathway of the [M-H]⁻ ion of both the compounds are reported in grey panels (theoretical *m/z* values are reported below each structure).



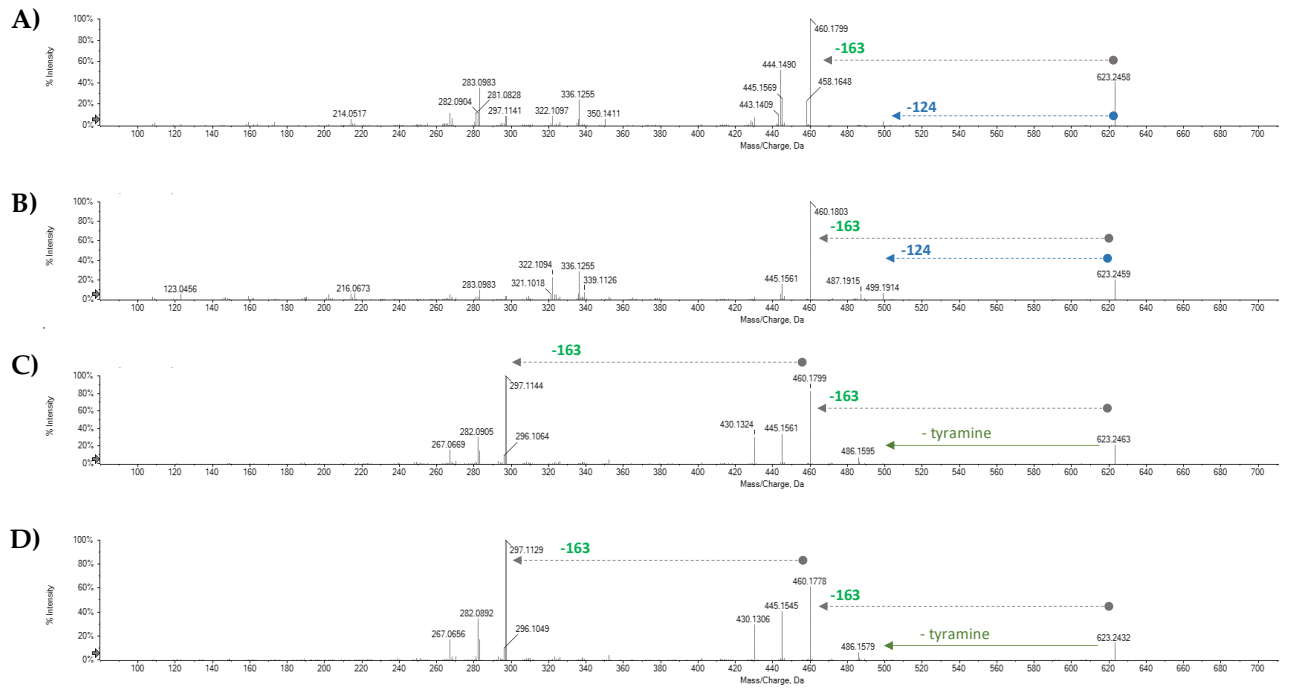


Figure 14. TOF-MS/MS spectra of compounds A) 27, B) 29, C) 37, and D) 39.

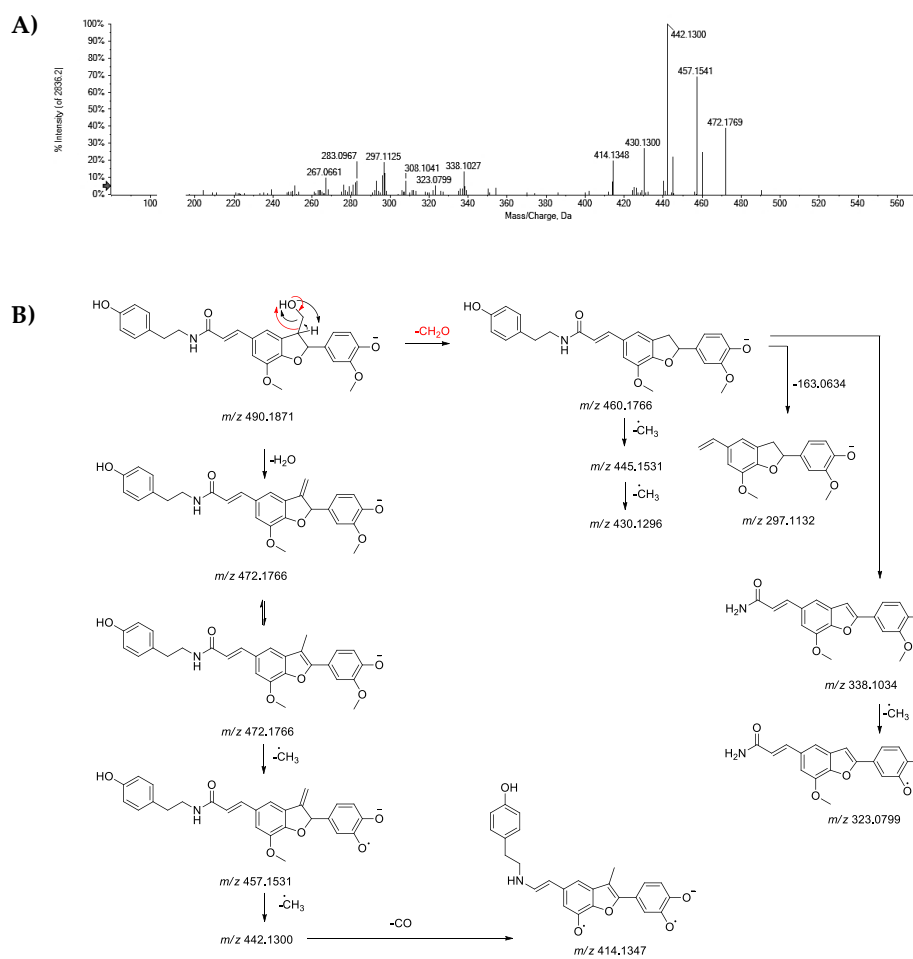


Figure 15. A) TOF-MS/MS spectrum of compound **30**, and B) proposed fragmentation pathway of its $[M-H]^-$ ion (theoretical m/z values are reported below each structure).

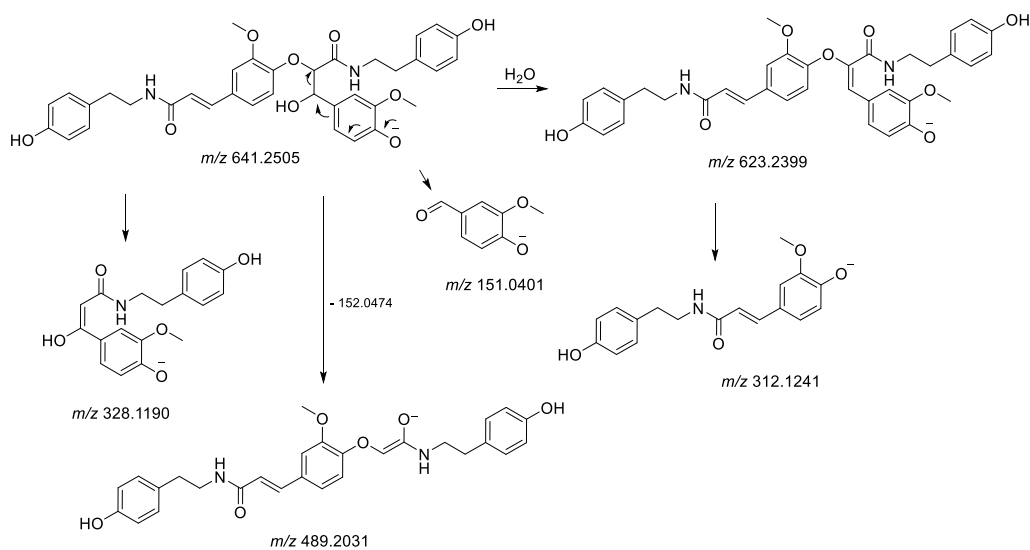
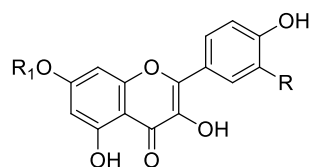


Figure 16. Proposed fragmentation pathway of the $[M-H]^-$ ion for compounds **31** and **34**.



- 3** R = OH; R₁ = pentose
- 7** R = OH; R₁ = deoxyhexose
- 9** R = H; R₁ = pentose
- 10** R = H; R₁ = pentose
- 12** R = H; R₁ = deoxyhexose
- 14** R = H; R₁ = deoxyhexose
- 18** R = OH; R₁ = acetyldeoxyhexose

Figure 17. Flavanol glycosides in LnHS hempseed fraction.

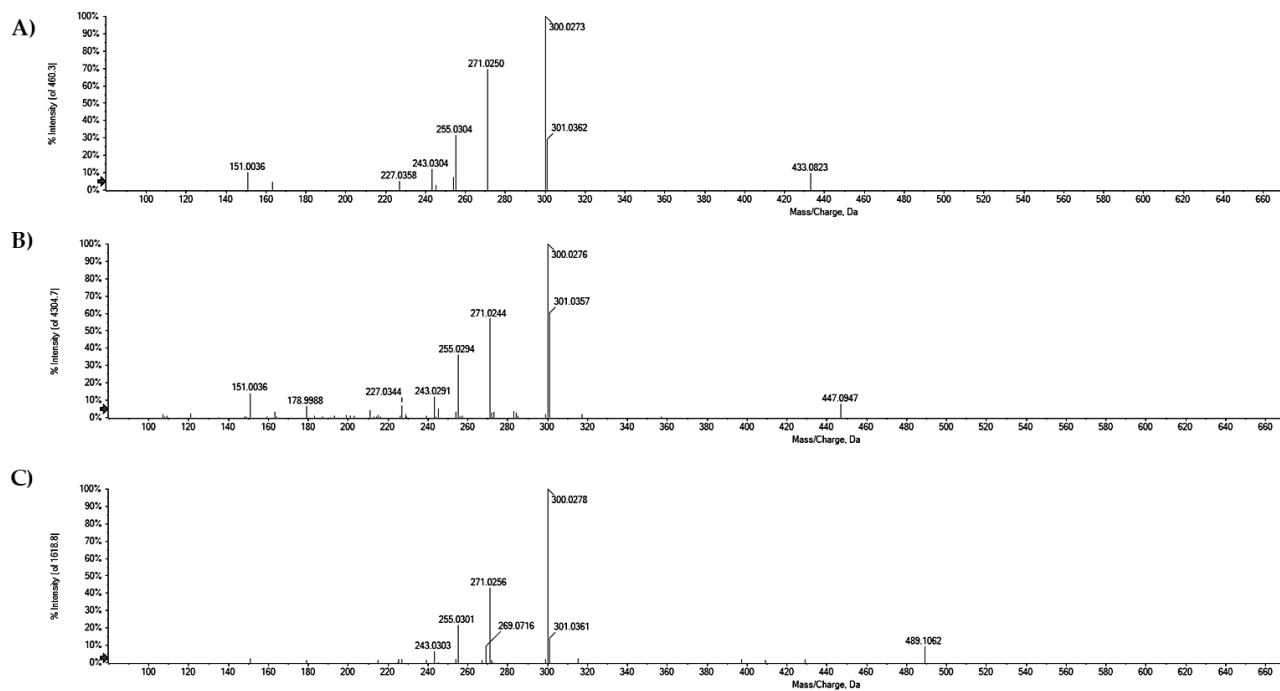


Figure 18. TOF-MS/MS spectra of quercetin derivatives A) 3, B) 7 and C) 18.

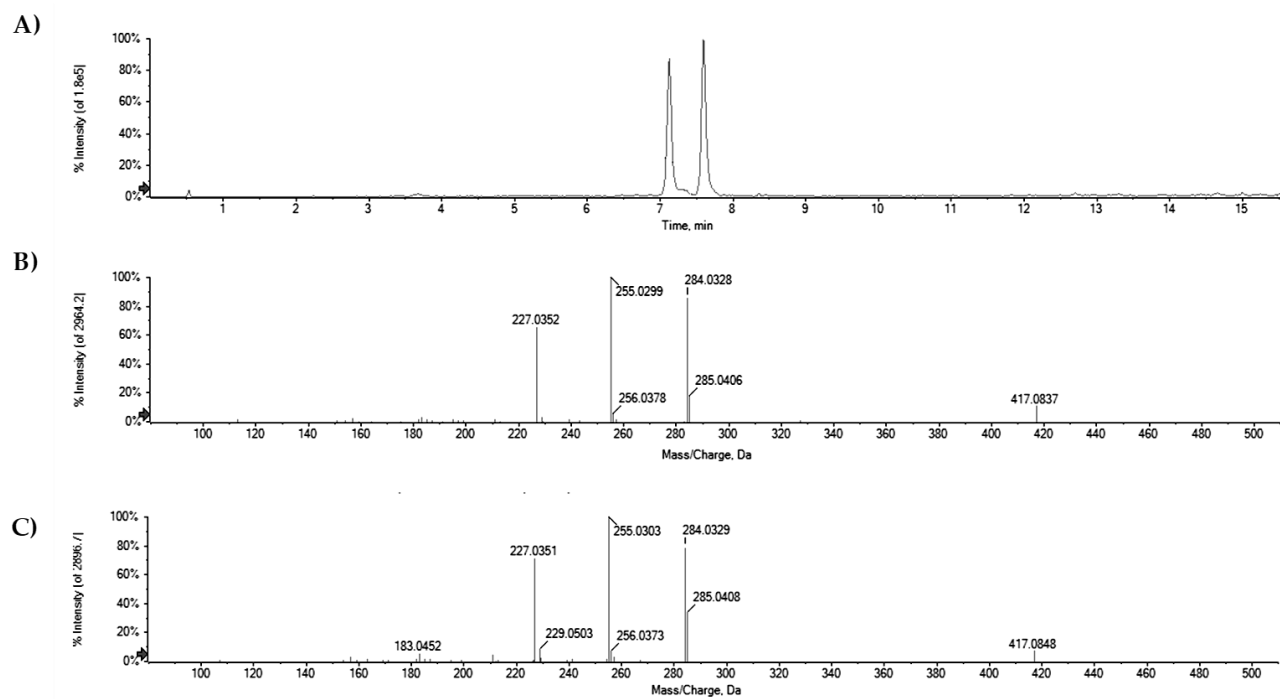


Figure 19. A) Extracted ion chromatogram (XIC) of the $[M-H]^-$ ion at m/z 417.083 ± 0.025 ; TOF-MS/MS spectra of B) compound **9**, and C) compound **10**.

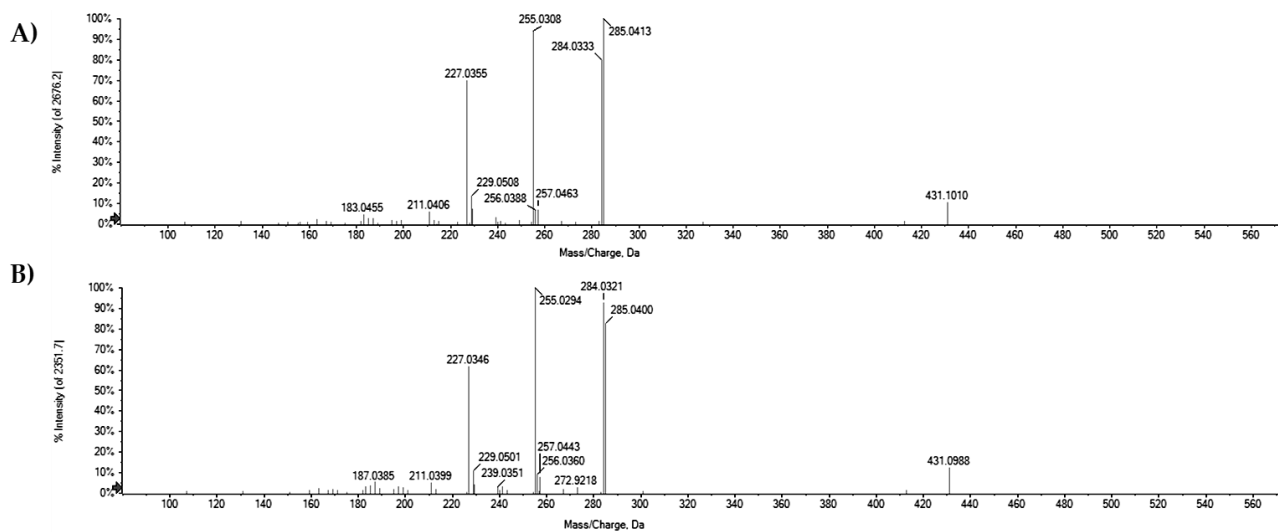


Figure 20. TOF-MS/MS spectra of kaempferol derivatives A) 12, and B) 14.

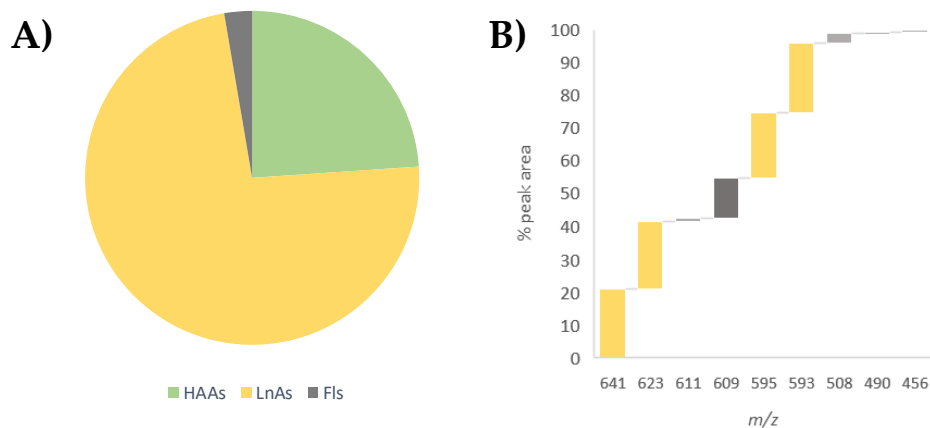


Figure 21. A) Relative content of each class of the tentatively identified compounds: HAAs – hydroxycinnamoyl amides; LnAs – lignanamides; Fls – Flavonols; B) Relative content of lignanamides sharing a common [M-H] ion in LnHS fraction.

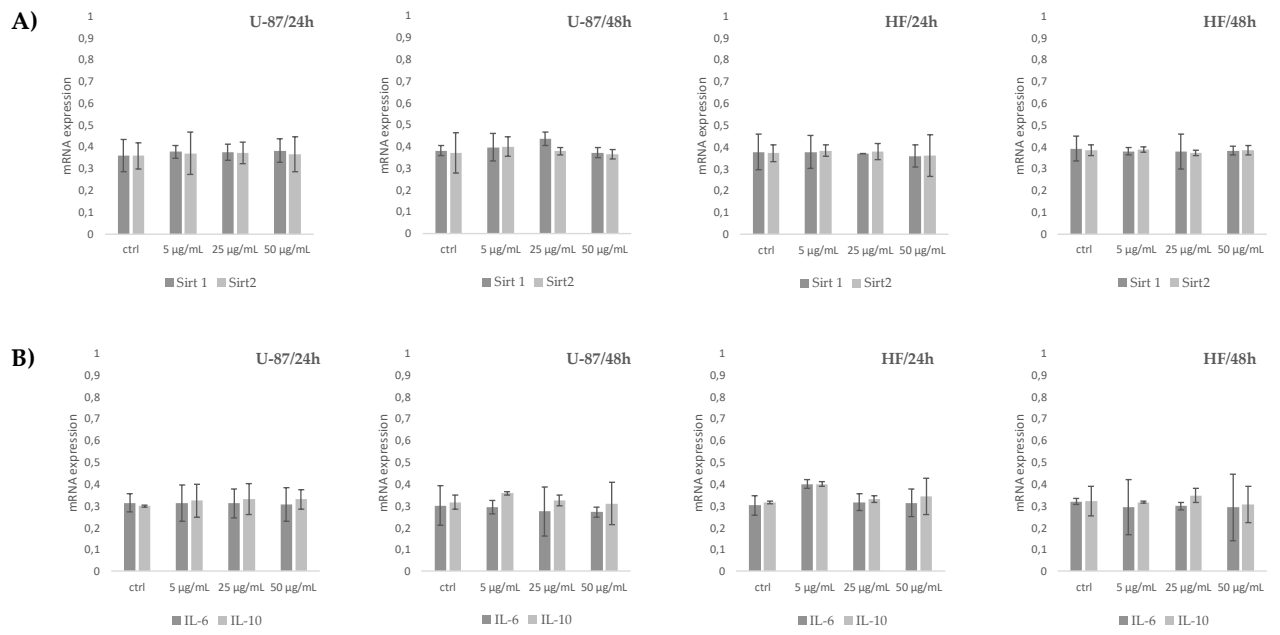


Figure 22. A) mRNA expression of Sirt1 and Sirt2 by real-time; B) mRNA expression of IL6 and IL10 by real-time in U-87 and human fibroblast (HF) cells after 24 and 48 h exposure times.