Supplementary Information

Safety evaluation of *Plukenetia volubilis* seeds: A metabolomic profiling and network toxicology approach

Contents

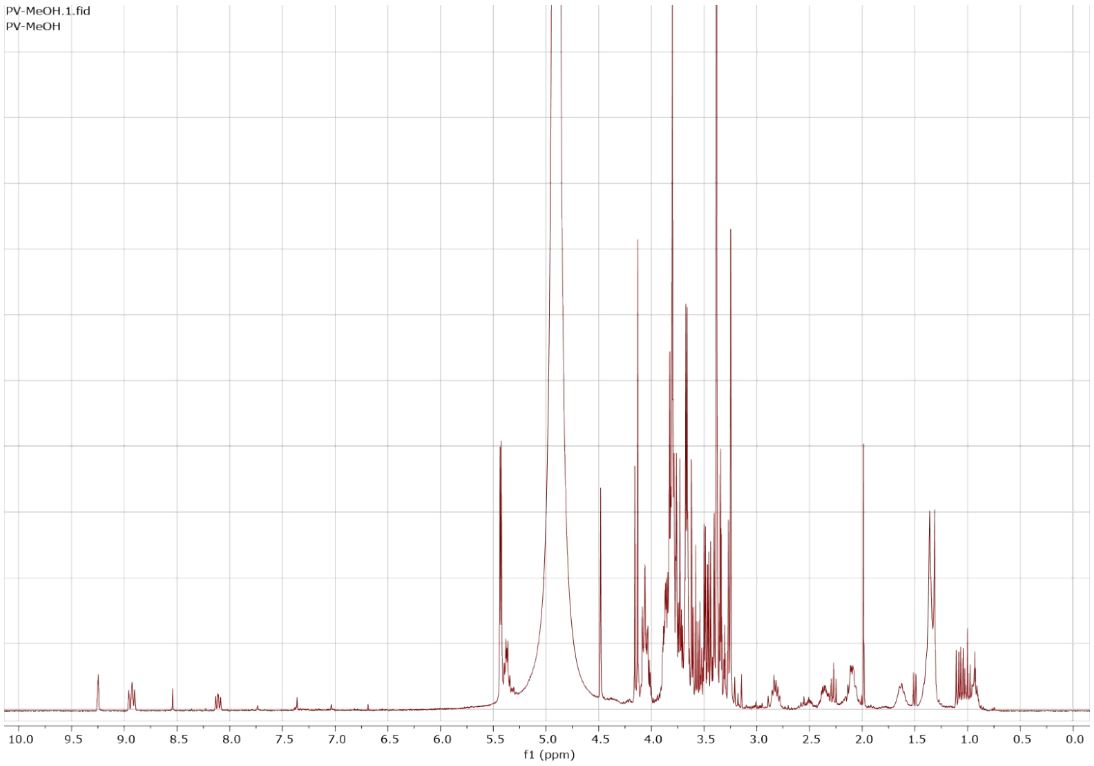

Figure S1. ^{1}H NMR spectrum (300 MHz) of MeOH extract in MeOH-d_4

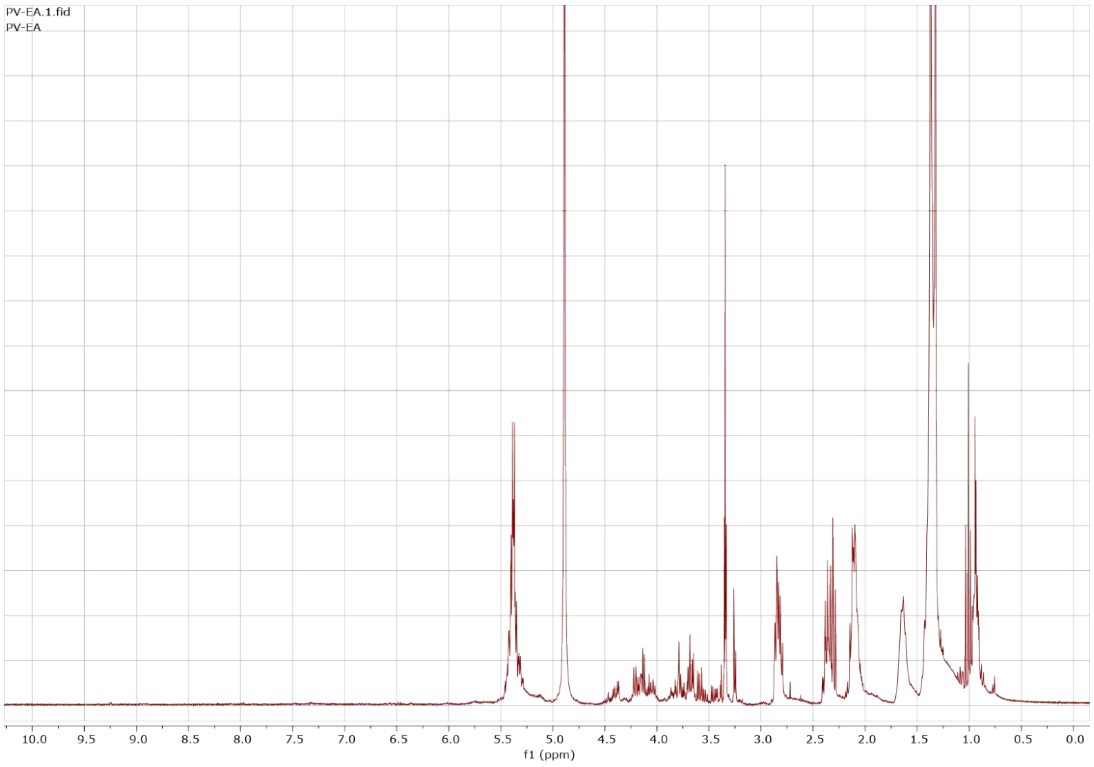

Figure S2. ^{1}H NMR spectrum (300 MHz) of EtOAc layer in MeOH-d_4

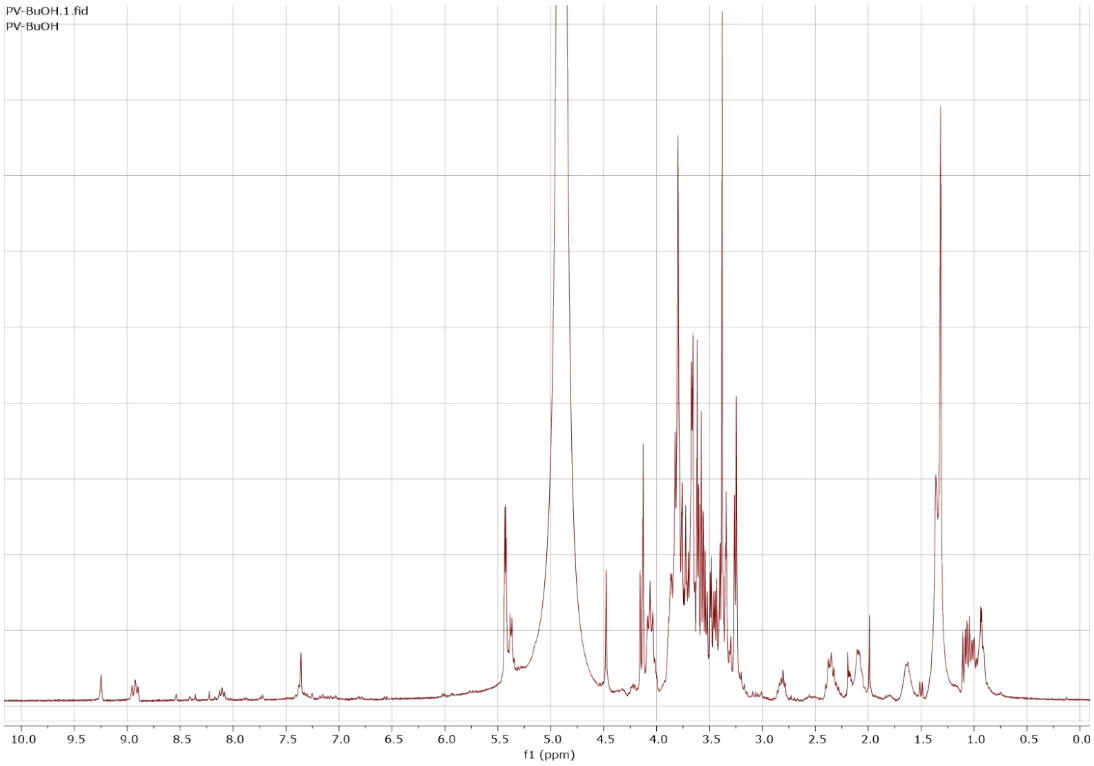

Figure S3. ^{1}H NMR spectrum (300 MHz) of *n*-BuOH layer in MeOH-d_4

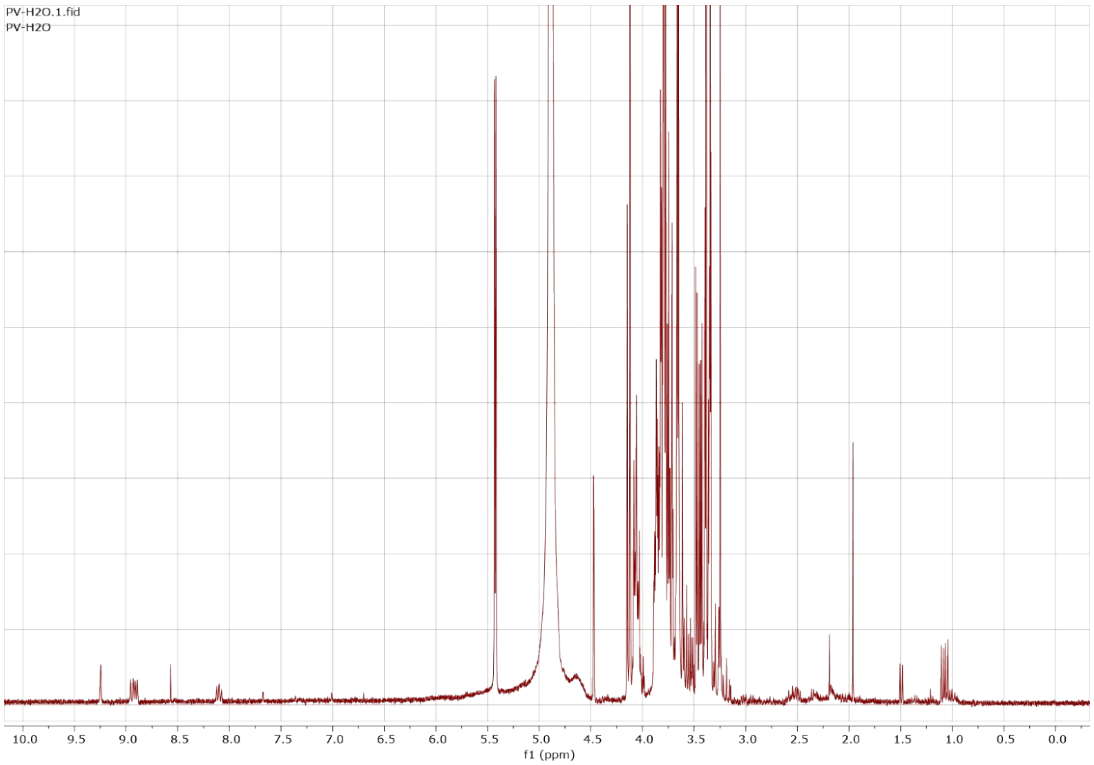

Figure S4. ^{1}H NMR spectrum (300 MHz) of H_2O layer in MeOH-d_4

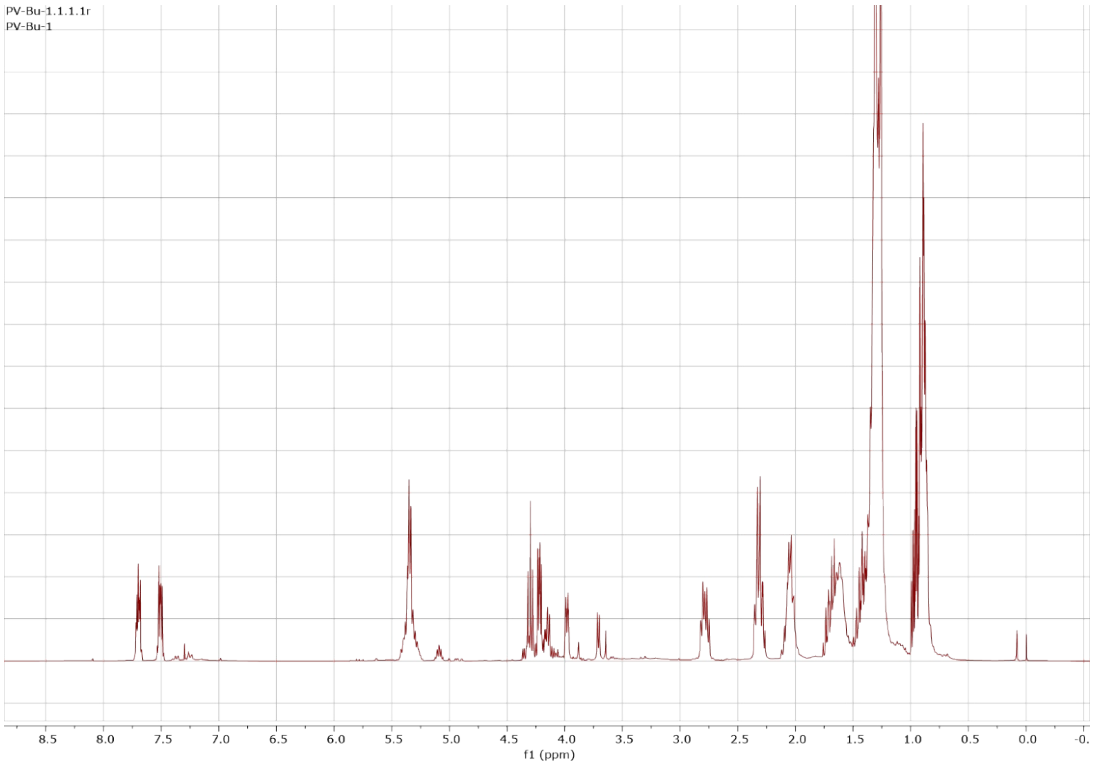

Figure S5. ^{1}H NMR spectrum (300 MHz) of fraction PV-Bu-1 in $CDCl_3$

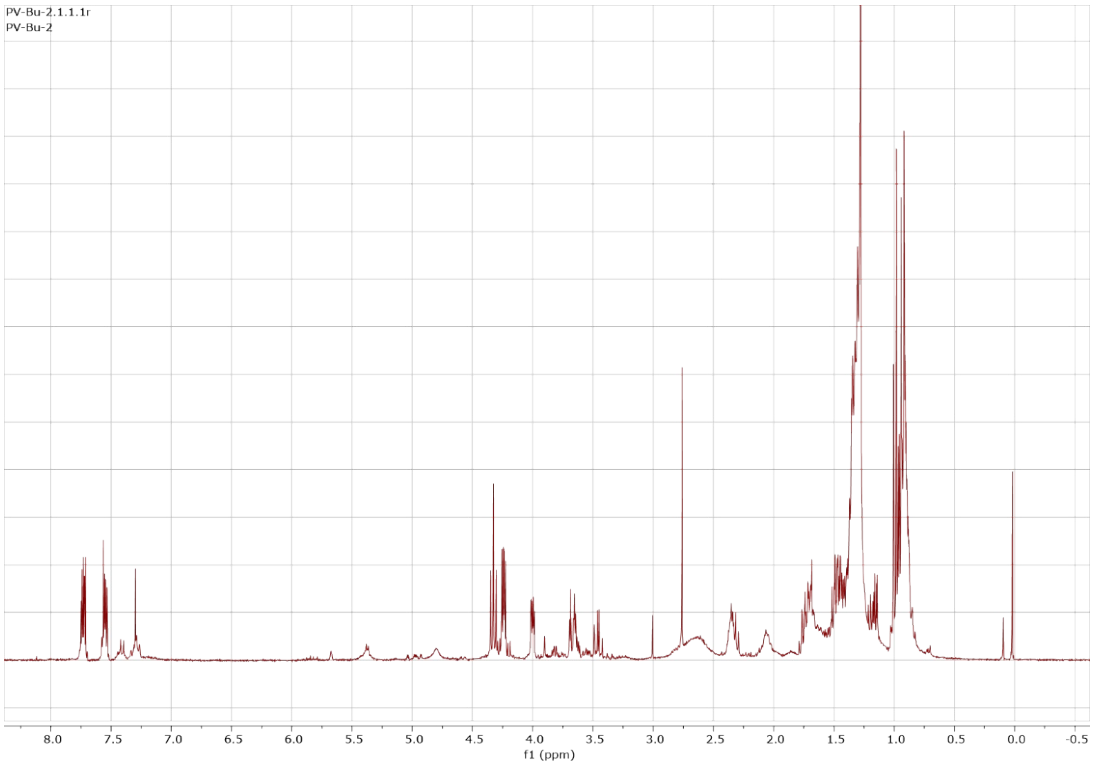

Figure S6. ^{1}H NMR spectrum (300 MHz) of fraction PV-Bu-2 in $CDCl_3$

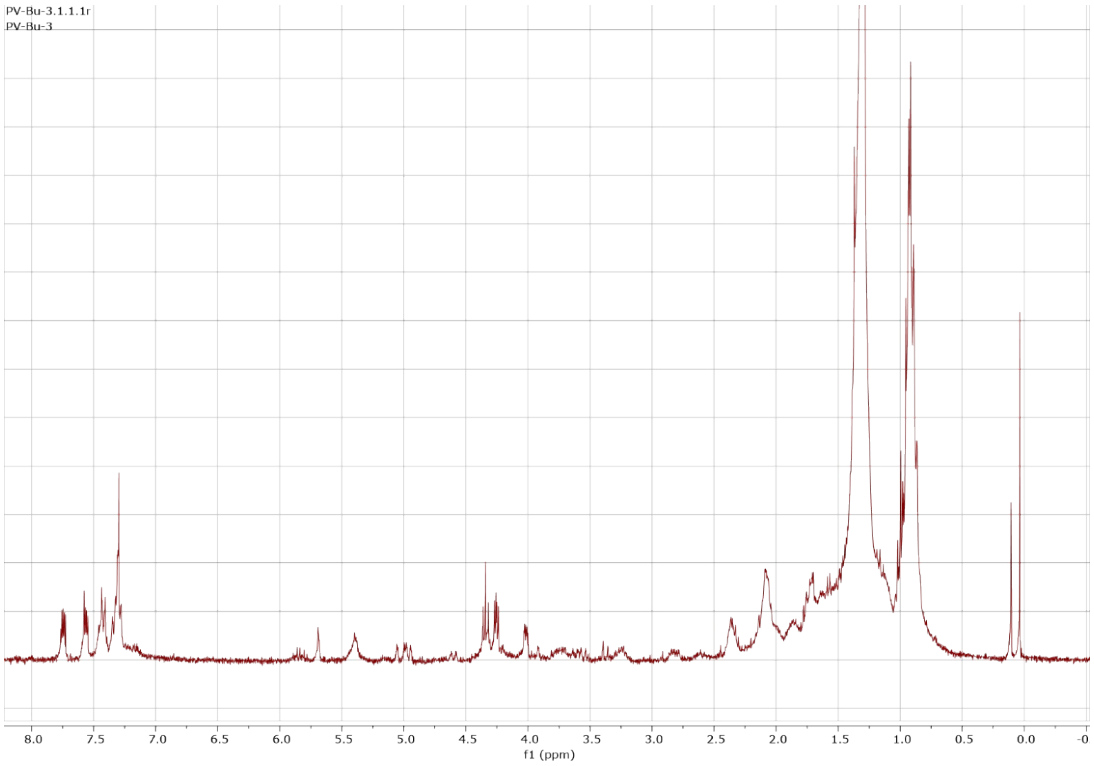

Figure S7. ^{1}H NMR spectrum (300 MHz) of fraction PV-Bu-3 in $CDCl_3$

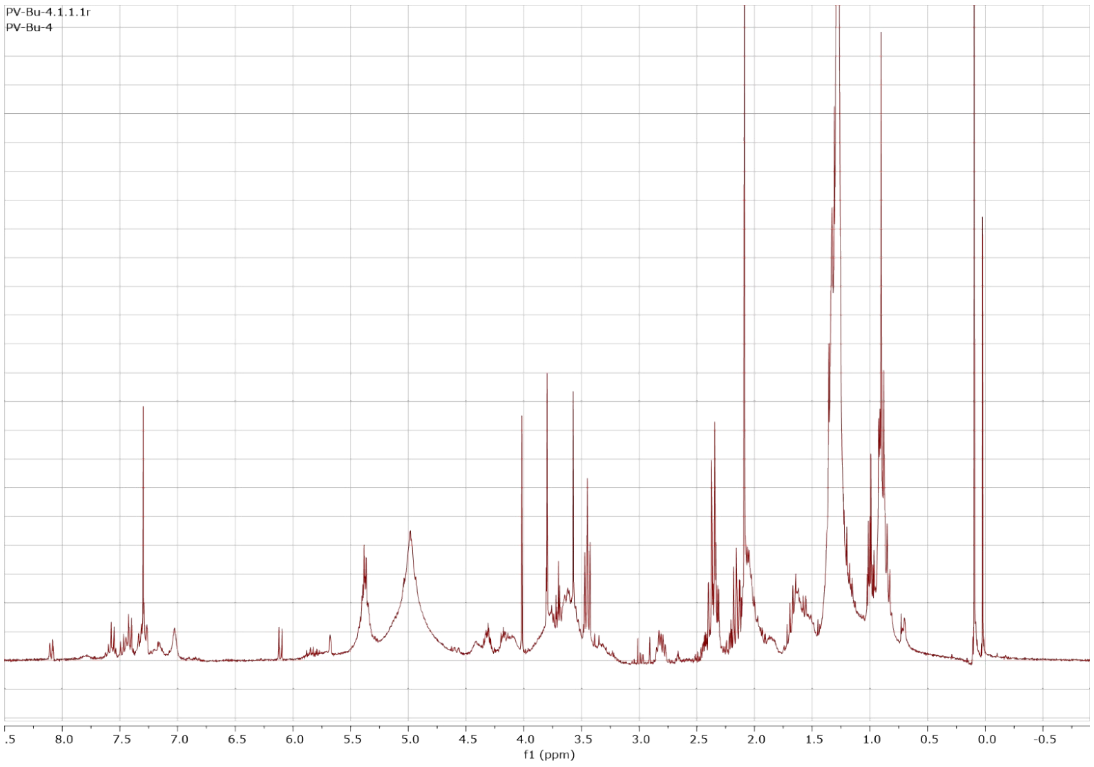

Figure S8. ^{1}H NMR spectrum (300 MHz) of fraction PV-Bu-4 in $CDCl_3$

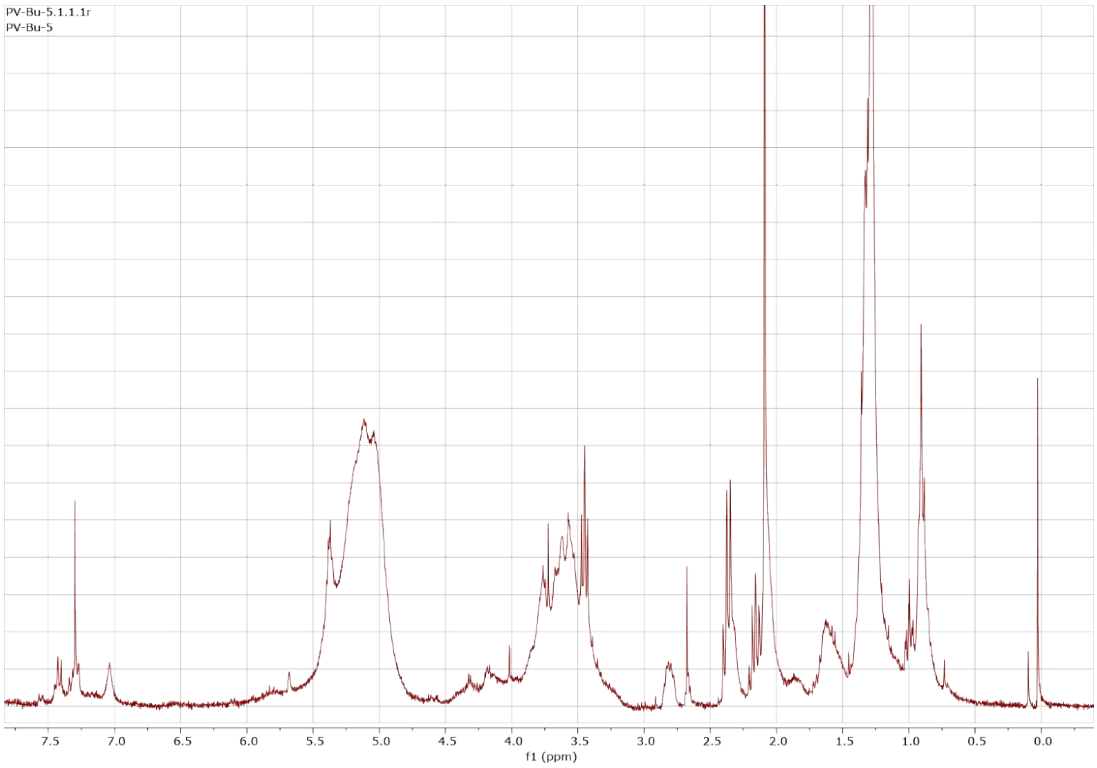

Figure S9. ^{1}H NMR spectrum (300 MHz) of fraction PV-Bu-5 in $CDCl_3$

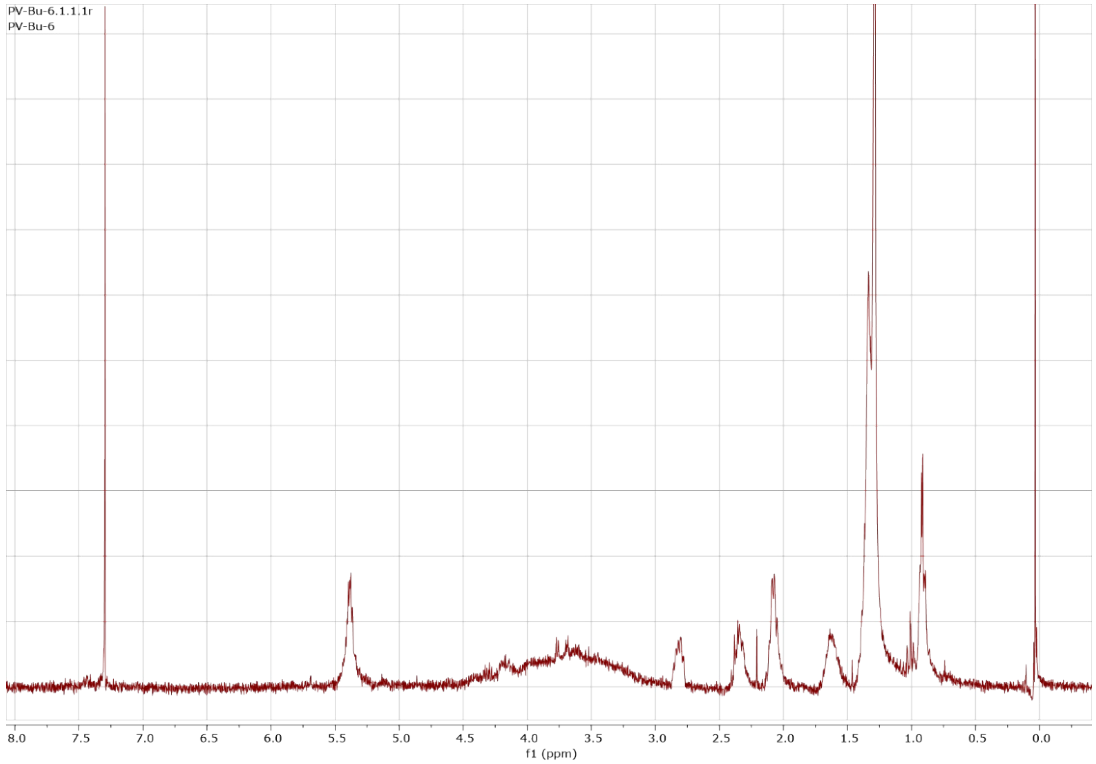

Figure S10. ^{1}H NMR spectrum (300 MHz) of fraction PV-Bu-6 in $CDCl_3$

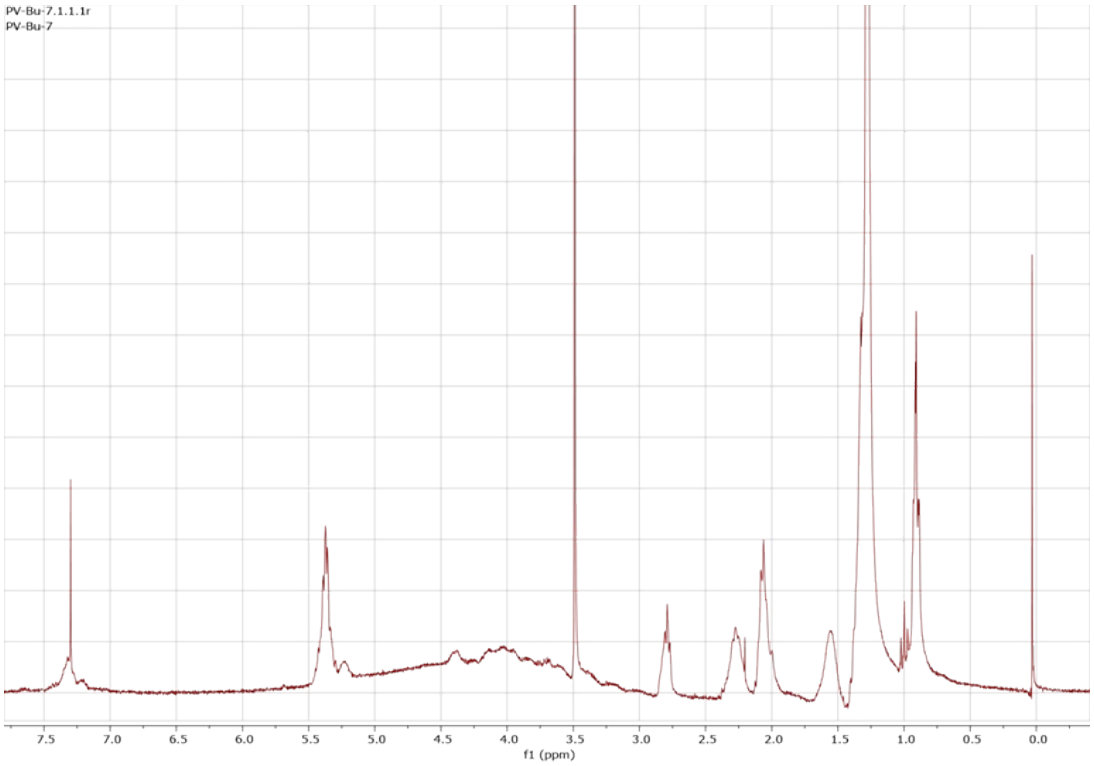

Figure S11. ^{1}H NMR spectrum (300 MHz) of fraction PV-Bu-7 in $CDCl_3$

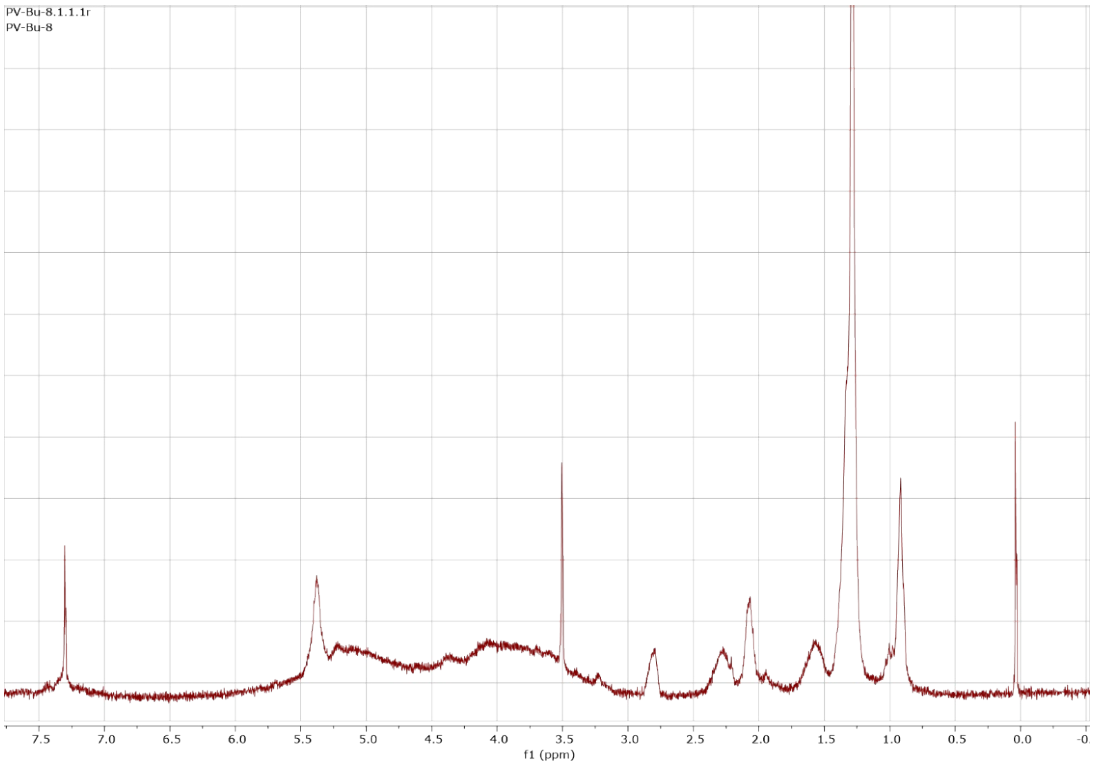

Figure S12. ^{1}H NMR spectrum (300 MHz) of fraction PV-Bu-8 in $CDCl_3$

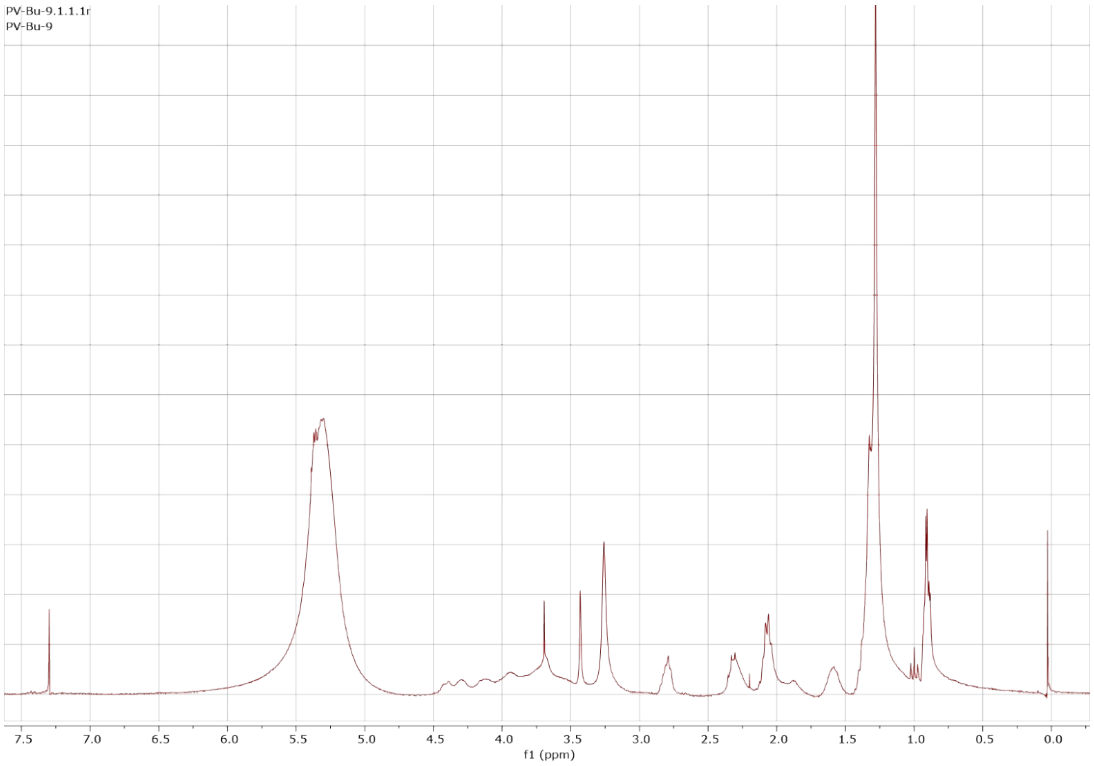

Figure S13. ^{1}H NMR spectrum (300 MHz) of fraction PV-Bu-9 in $CDCl_3$

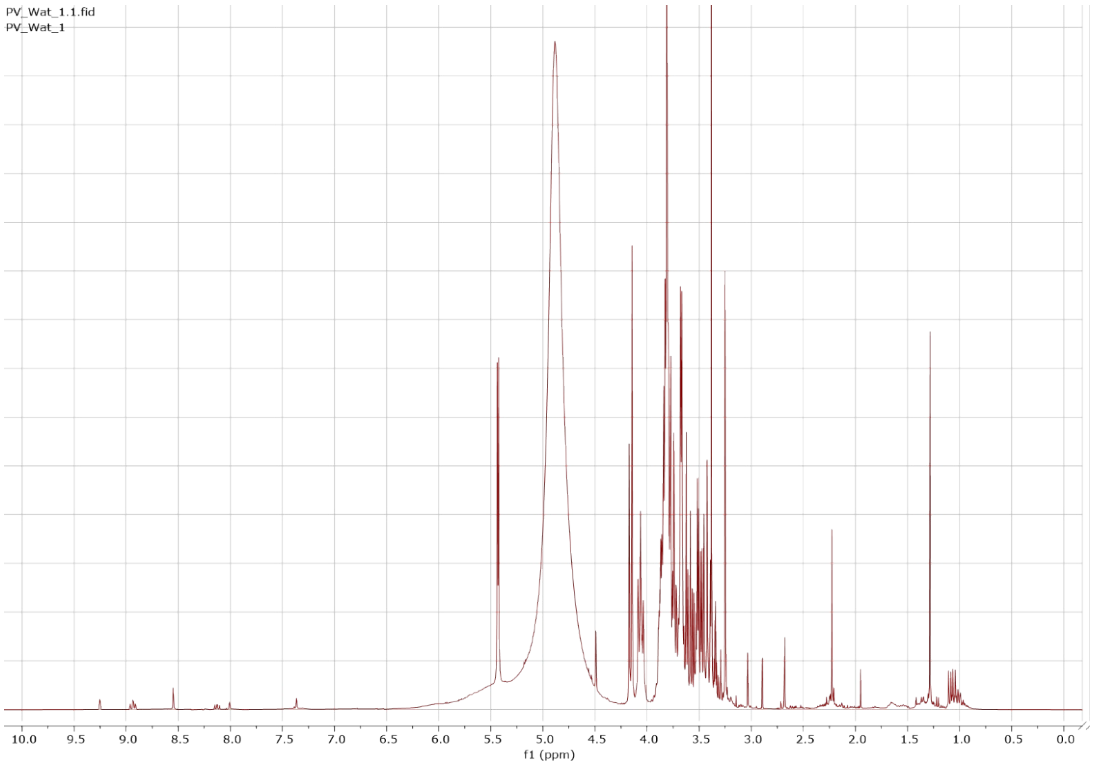

Figure S14. ^{1}H NMR spectrum (300 MHz) of fraction PV-H_2O-1 in MeOH-d_4

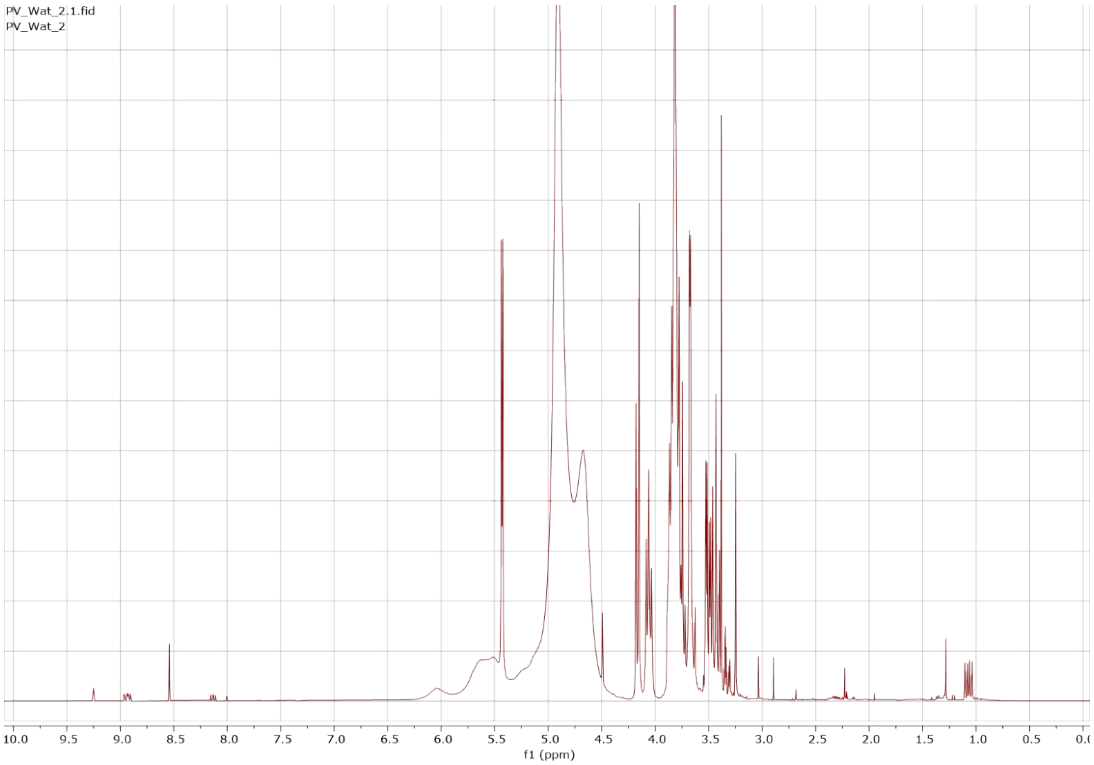

Figure S15. ^{1}H NMR spectrum (300 MHz) of fraction PV-H_2O-2 in MeOH-d_4

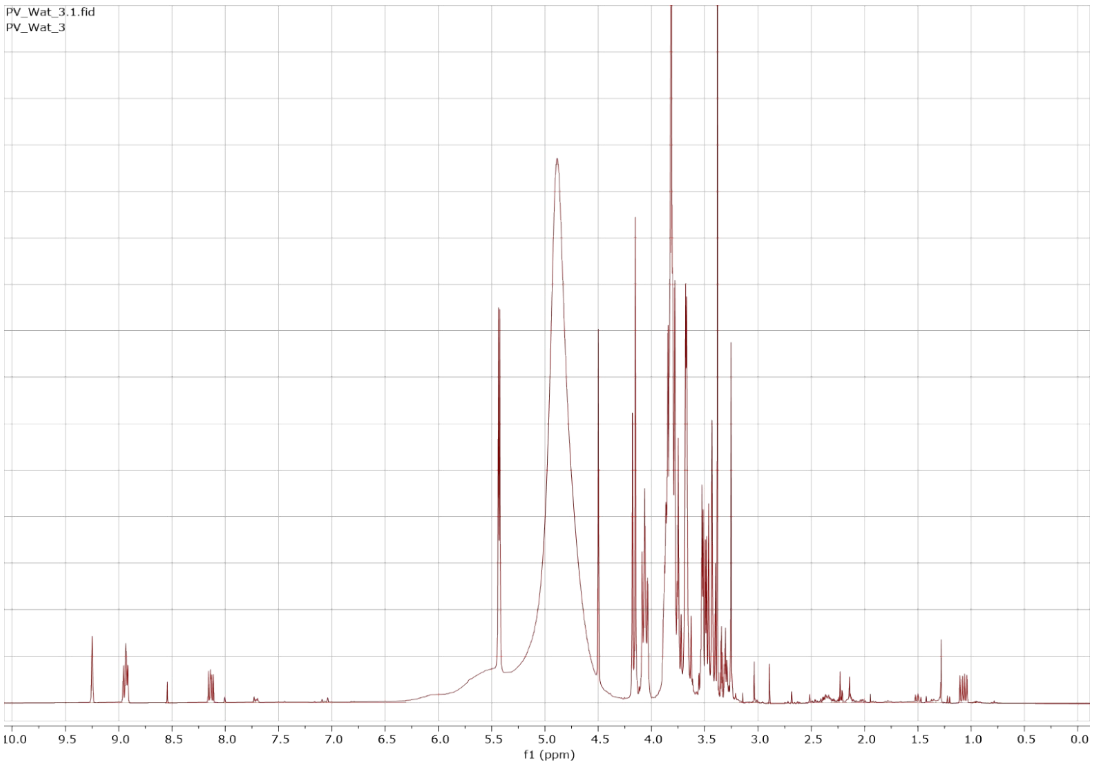

Figure S16. ^{1}H NMR spectrum (300 MHz) of fraction PV-H_2O-3 in MeOH-d_4

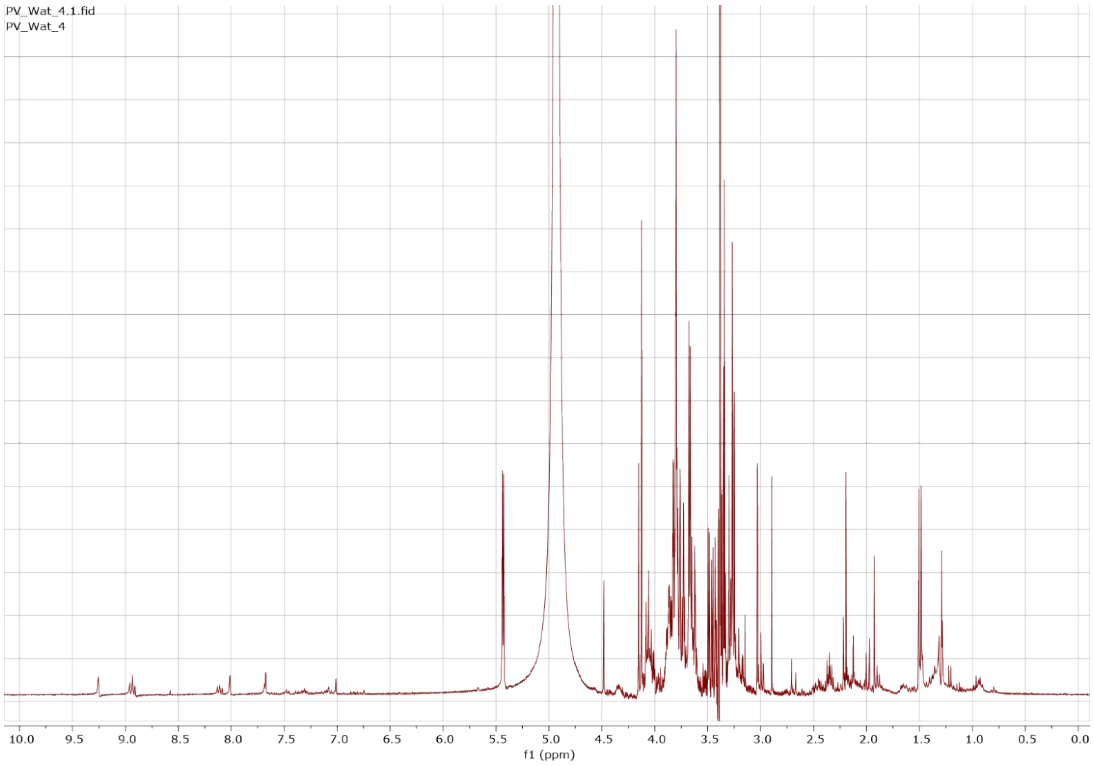

Figure S17. ^{1}H NMR spectrum (300 MHz) of fraction PV-H_2O-4 in MeOH-d_4

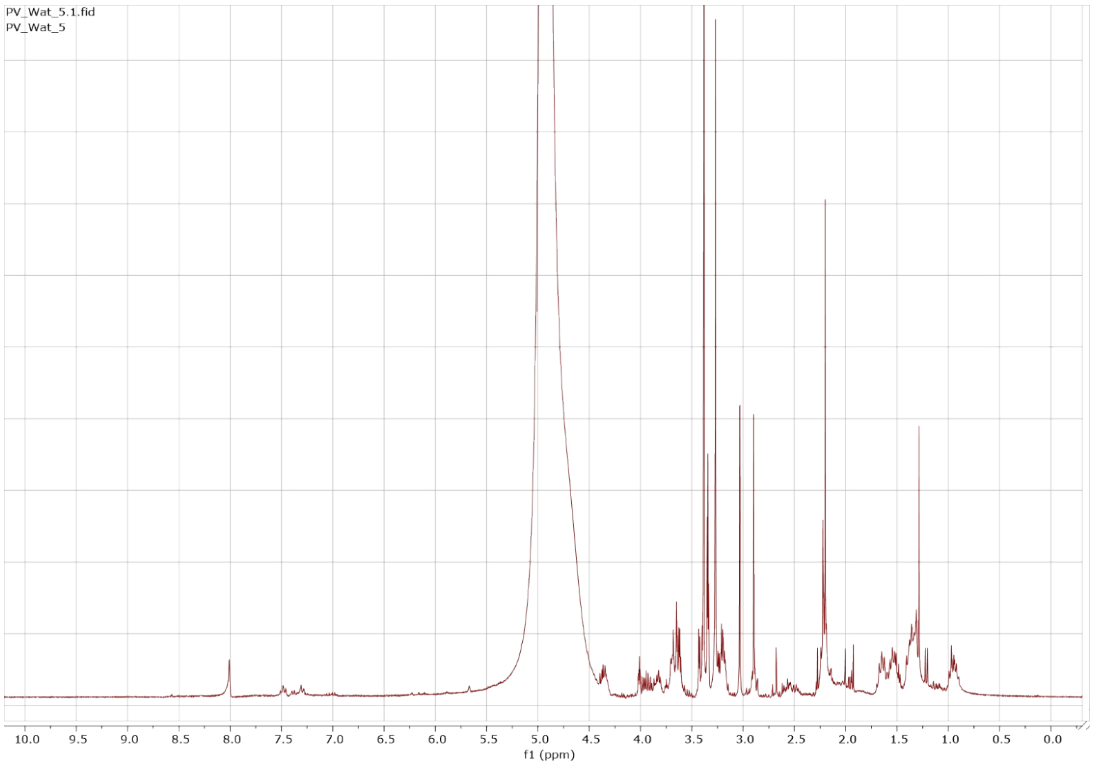

Figure S18. ^{1}H NMR spectrum (300 MHz) of fraction PV-H_2O-5 in MeOH-d_4

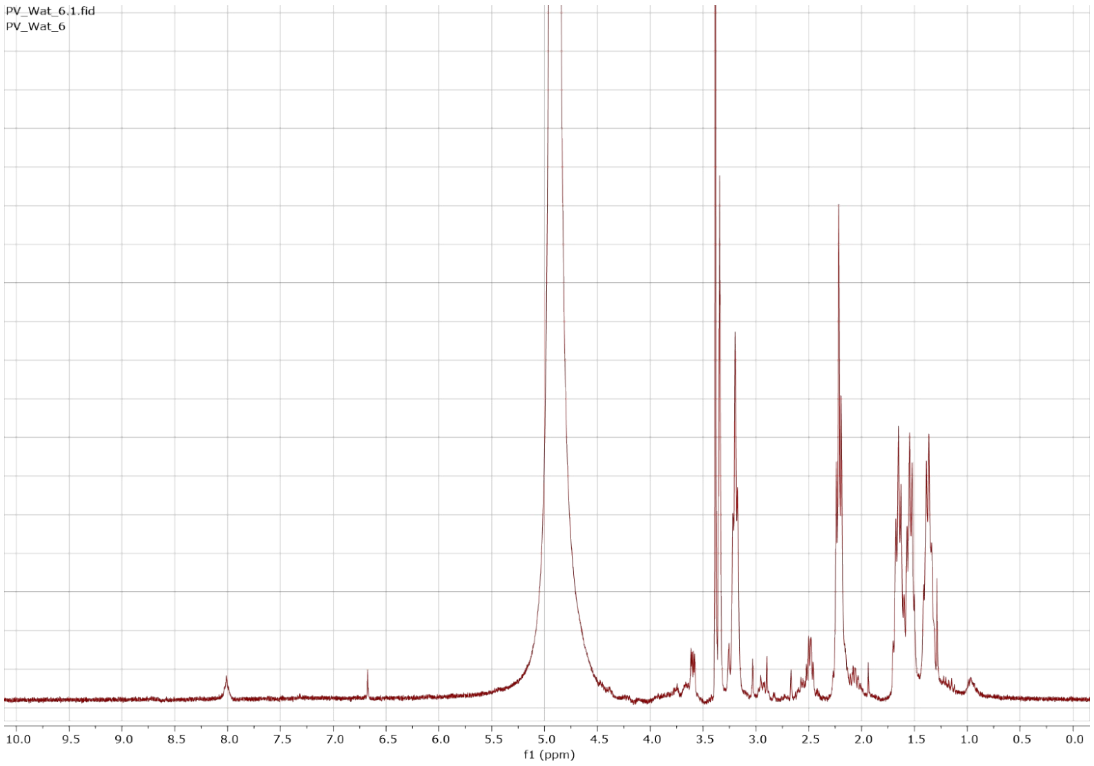

Figure S19. ^{1}H NMR spectrum (300 MHz) of fraction PV-H_2O-6 in MeOH-d_4

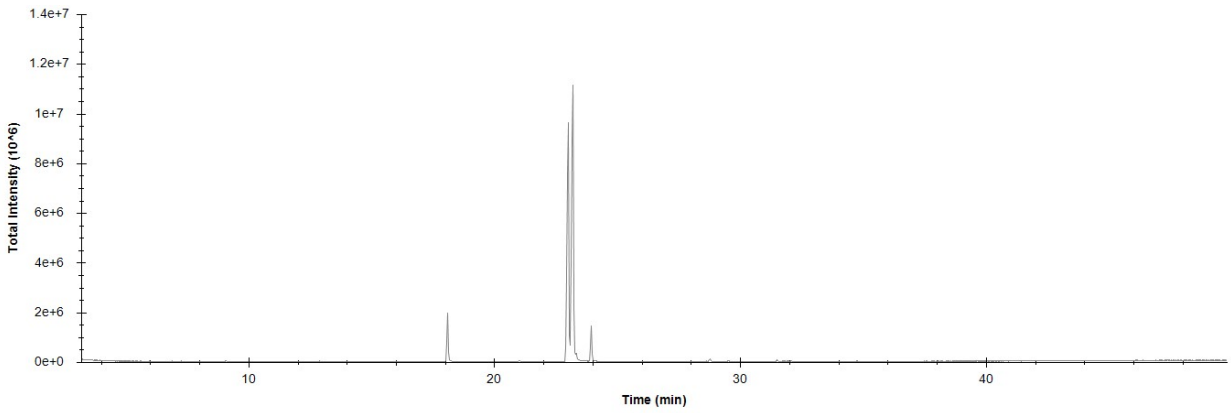

Figure S20. GC-MS spectrum of the SI oil FAMEs

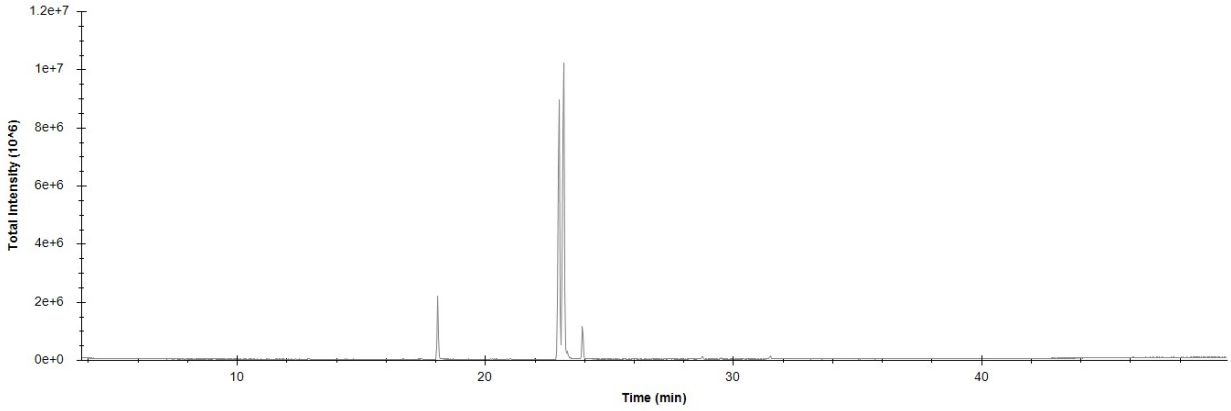

Figure S21. GC-MS spectrum of the n-hexane layer FAMEs

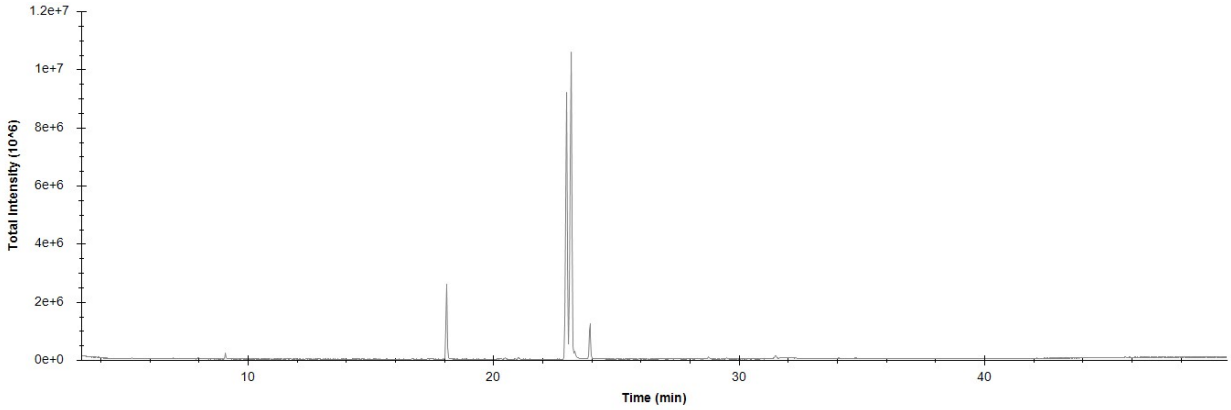

Figure S22. GC-MS spectrum of the EtOAc layer FAMEs

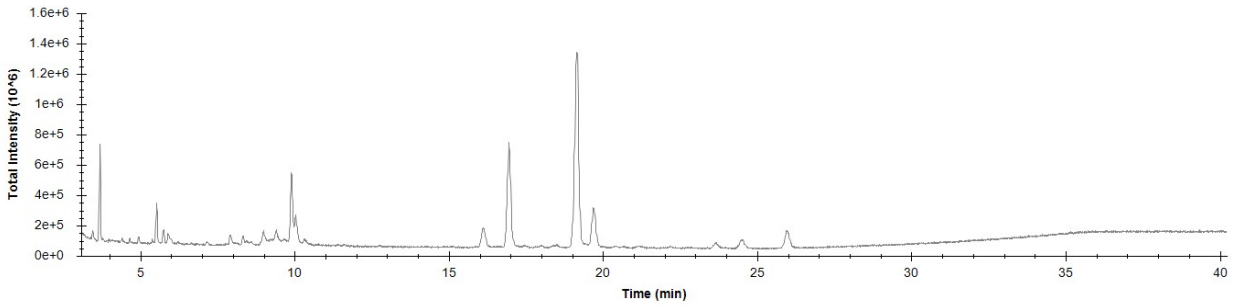

Figure S23. GC-MS spectrum of the saponified SI oil

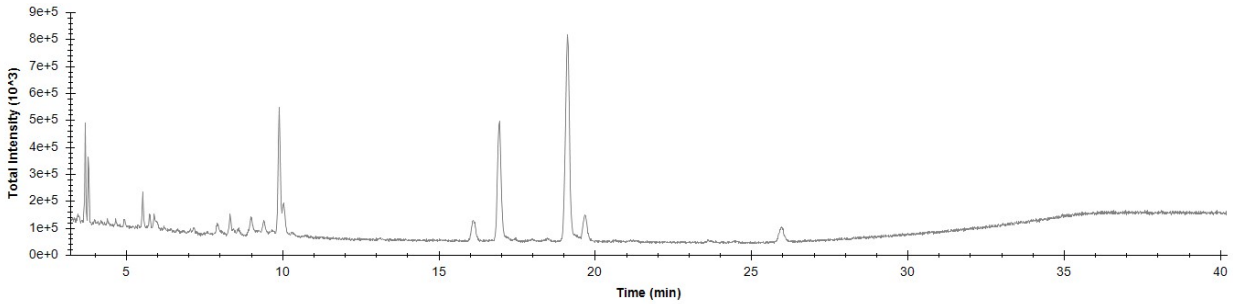

Figure S24. GC-MS spectrum of the saponified n-hexane layer

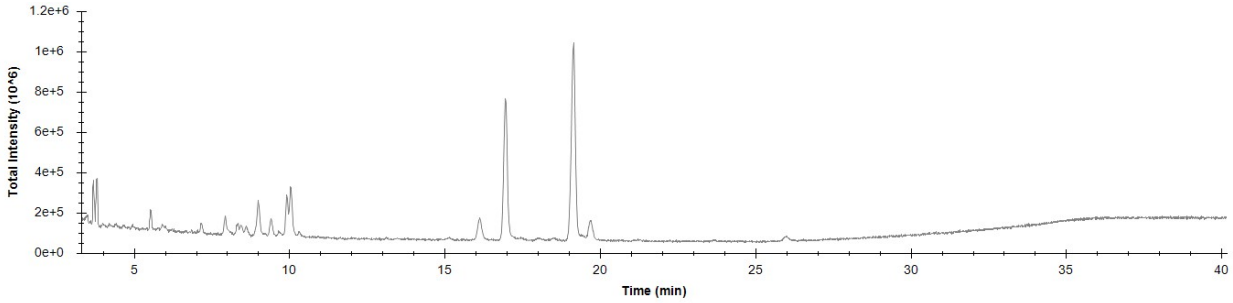

Figure S25. GC-MS spectrum of the saponified EtOAc layer

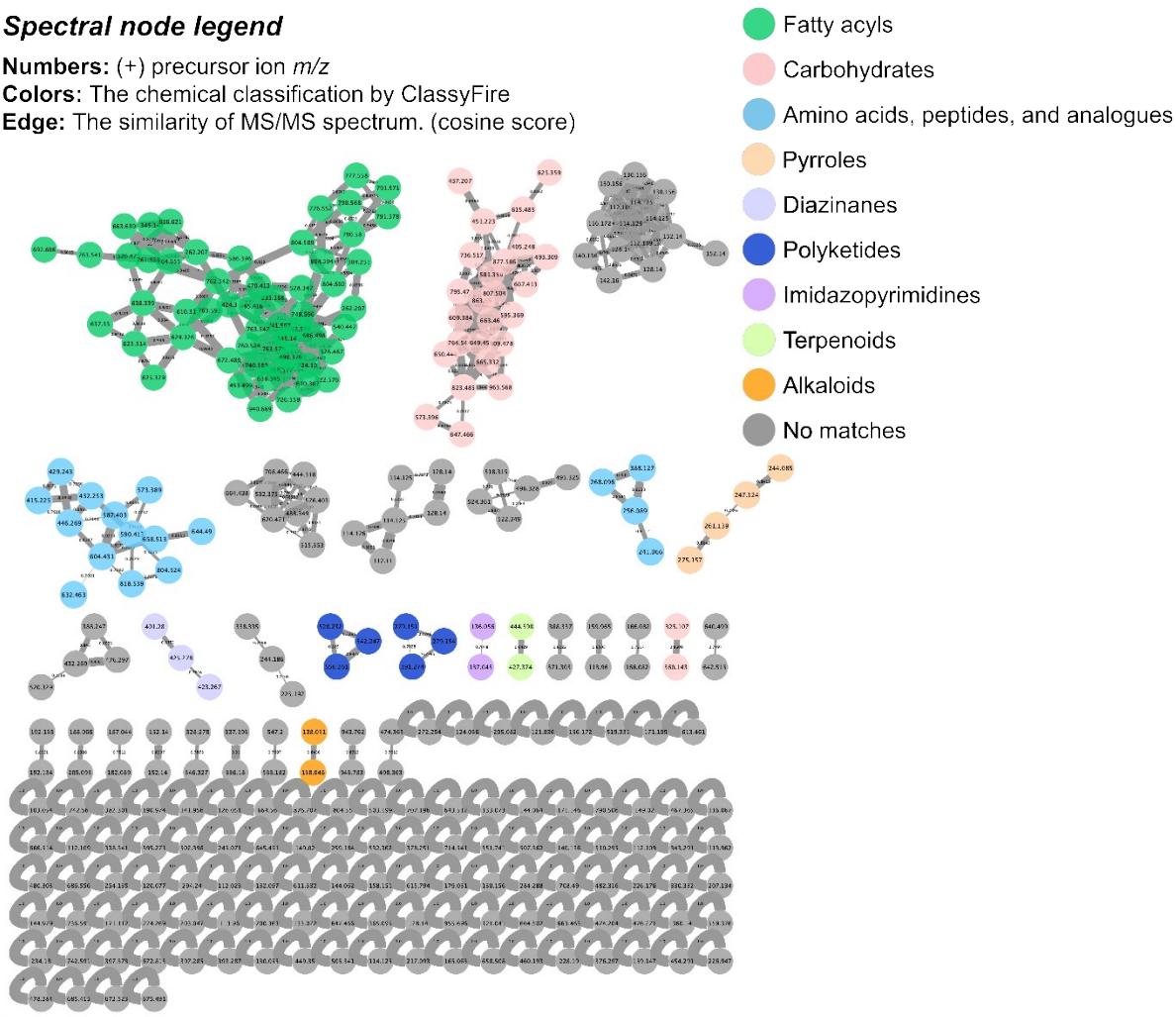

Figure S26. The metabolomic classification of fractions PV-Bu-1–9

Spectral node legend

Numbers: (+) precursor ion *m/z*
Colors: The chemical classification by ClassyFire
Edge: The similarity of MS/MS spectrum. (cosine score)

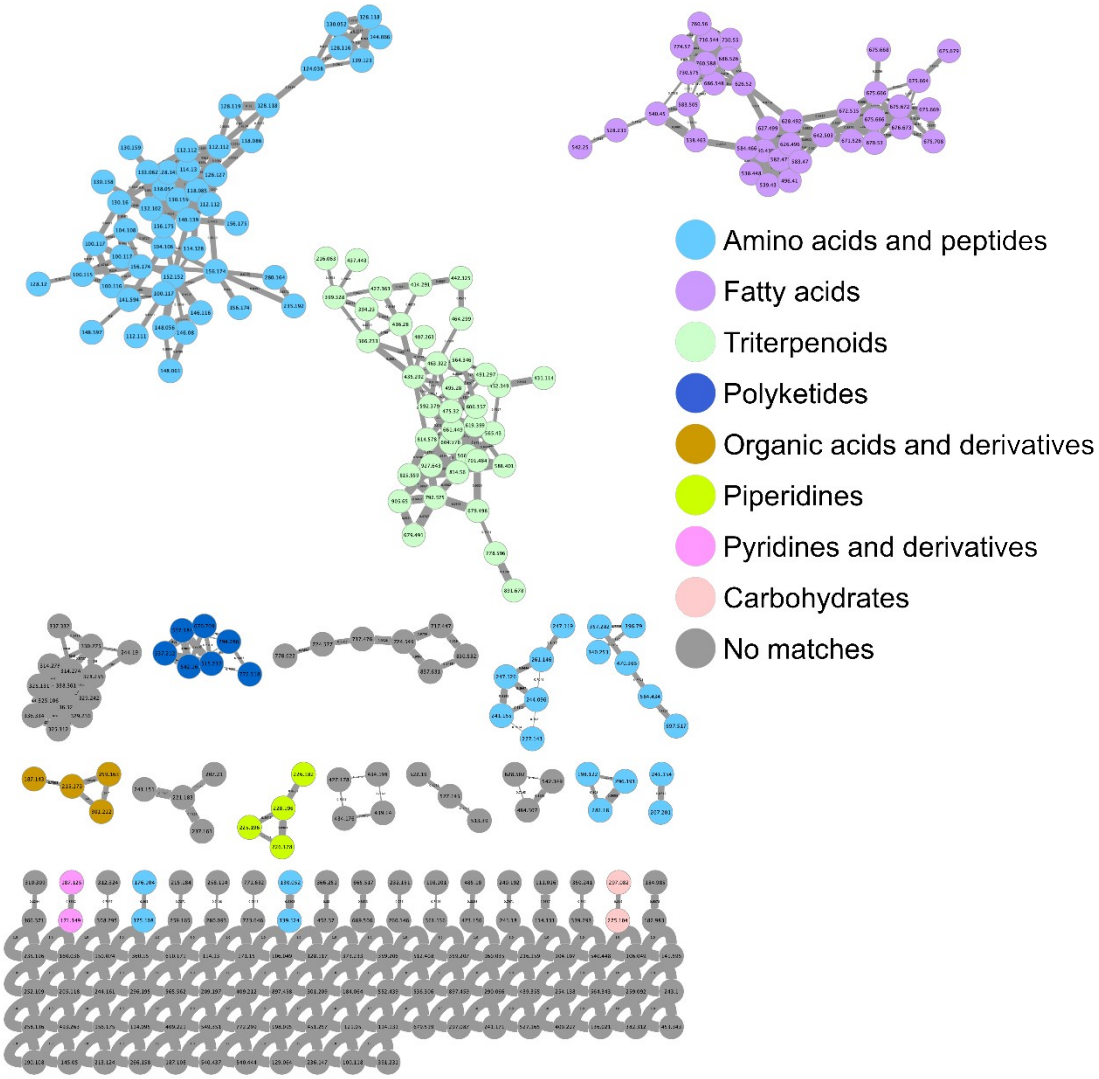

Figure S27. The metabolomic classification of fractions PV-H_2O-1–6

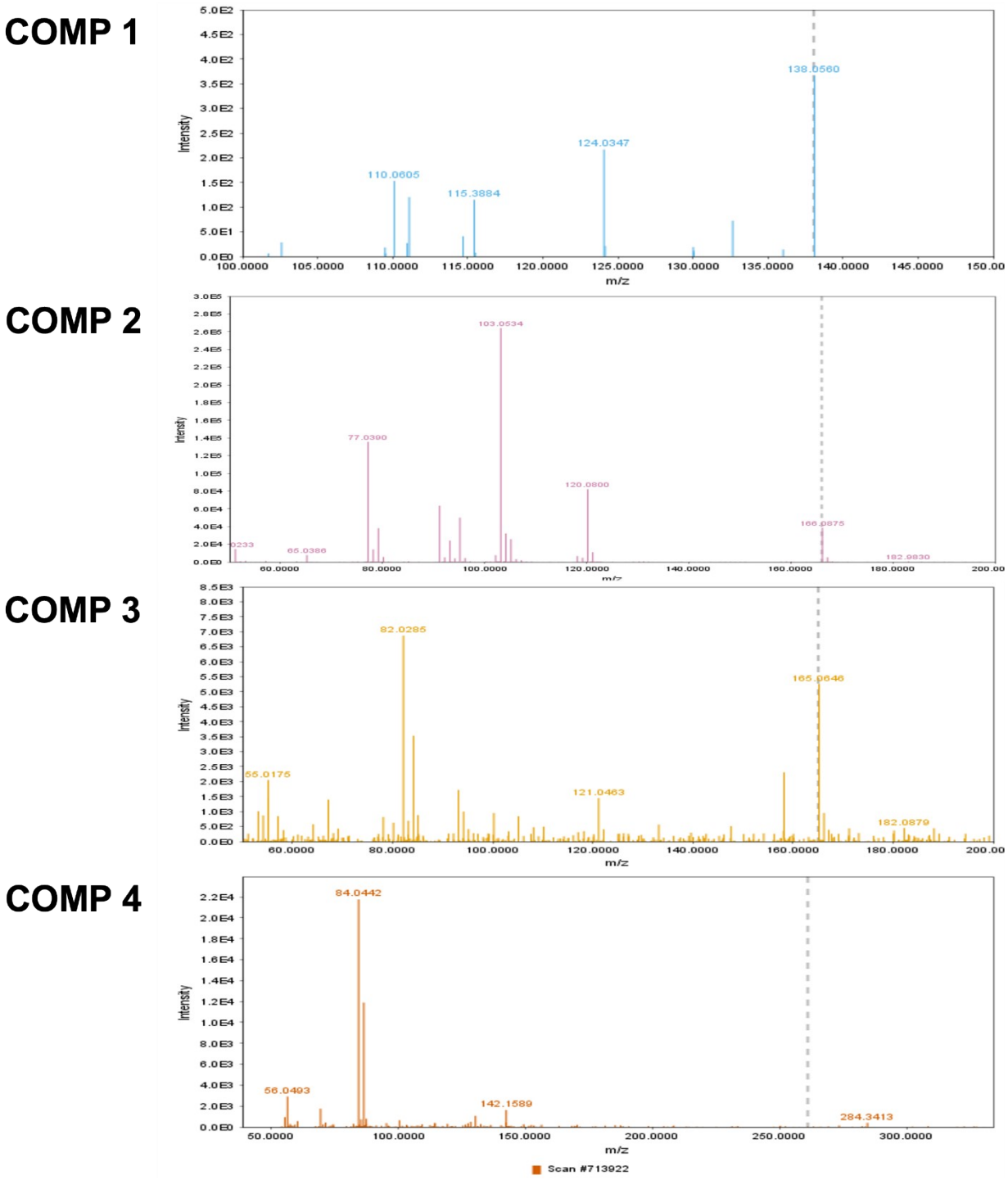

Figure S28. The MS^2 spectra of compounds **1**–**4**

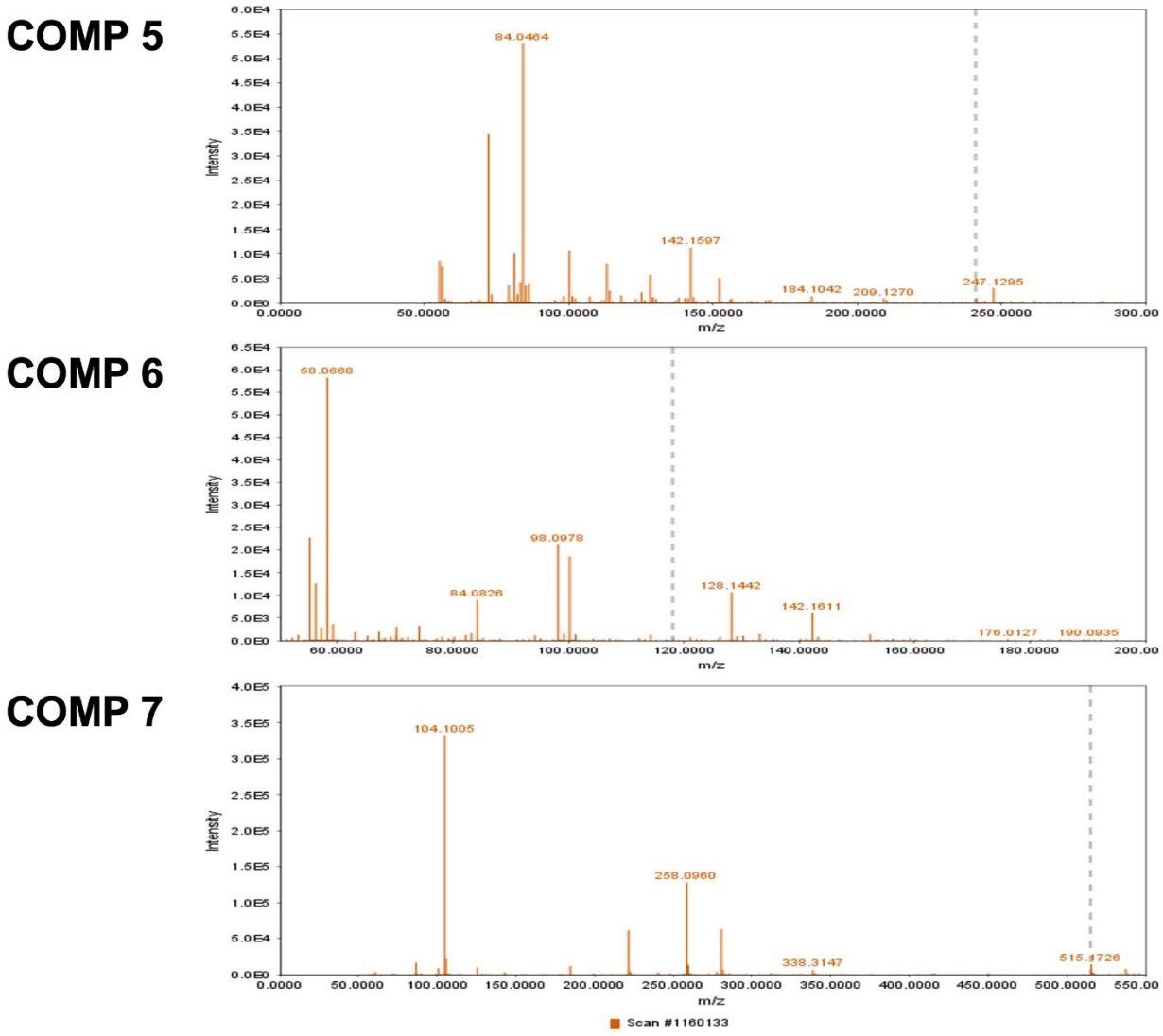

Figure S29. The MS^2 spectra of compounds **5**–**7**

Detailed parameters used in the MZmine software:

Peak Detection Noise level: MS1 1.0E4, MS2 1.0E3 # ADAP chromatogram builder Min group size: 3; Group intensity threshold: 1.0E3; min highest intensity: 2E2; *m*/*z* tolerance: 0.02 Da or 20 ppm # Chromatogram Deconvolution – Wavelets (ADAP) S/N threshold: 10; min feature height: 100; *m*/*z* range for MS2 scan pairing: 0.02 Da; RT range for MS2 scan paring: 0.15 min. Chromatogram Deconvolution - Algorithm: ADAP wavelets; *m*/*z* range for MS2 scan pairing: 0.02 Da; RT range for MS2 scan paring: 0.15 min; S/N threshold: 10; min feature height: 100; coefficient/area threshold: 50; Peak duration range: 0.05-1.00; RT wavelet range: 0.03-0.15 # isotopic peak grouper *m*/*z* tolerance: 0.01 Da or 10 ppm; RT tolerance: 0.1 min; Max charge: 2 # Group MS2 scans with features RT tolerance: 0.02 min; *m*/*z* tolerance: 0.01 Da or 10 ppm # Filter Keep only peaks with MS2 scan (GNPS) # Gap-Filling Intensity tolerance 20%; RT tolerance: 0.2 min for both; *m*/*z* tolerance: 0.02 Da or 20 ppm # GNPS export.

Table S1. Calibration curve, range, precision and accuracy of trigonelline

Linearity	Precision			Accuracy		
Calibration curve	Concentration (µg/mL)	Intraday (n = 6)	Interday (n = 3)	Spiked conc (µg/mL)	Recovery rate (%)	RSD (%)
Y = 20849X + 12735	40.23	RSD = 0.407%	RSD = 0.873%	7.8	110.3%	1.56%
R^2 = 0.99996				31.25	102.65%	0.41%
Range: 3.9–250 µg/mL				125	99.4%	0.08%

Retention time: 7.15 min.