ANTARCTIC SOIL METABOLOMICS: A PILOT STUDY

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Supplementary Materials

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Table S1. Physicochemical parameters of the Antarctic soils analysed in this study (data extracted from [21] - Severgnini, M.; Canini, F.; Consolandi, C.; Camboni, T.; Paolo D'Acqui, L.; Mascalchi, C.; Ventura, S.; Zucconi, L. Highly Differentiated Soil Bacterial Communities in Victoria Land Macro-Areas (Antarctica). *FEMS Microbiol. Ecol.* **2021**, *97*, fiab087. https://doi.org/10.1093/femsec/fiab087).

	N %	C%	C/N ratio	pH	Na (cmol/kg)	K (cmol/kg)	Mg (cmol/kg)	Ca (cmol/kg)	CEC (cmol/kg)	Soil Moisture %	Sand %	Coarse silt %	Fine silt %	Clay %
Ap.I 1	0.202	5.87	29.1	7.76	0.06	0.14	0.4	0.25	0.85	0.595	74.1	12.3	12.9	0.7
Ap.I 2	0.243	6.31	26											
B.By 1	0.085	2.07	24.4	8.32	0.07	0.33	1.44	2.43	3.93	0.71	70.4	4.1	18.4	7.2
B.By 2	0.035	0.88	25.14											
Cp.K 1	0.103	0.91	8.8	7.6	0.14	0.34	2.18	1.89	6.42	0.432	93.7	2.5	3	0.8
Cp.K 2	0.066	1.87	28.33							0.35				
Ed.P 2	0.028	0.565	2.54	6.595	0.42	0.29	0.22	0.37	1.29	0.176	81	8.6	9.6	0.9
Ed.P 3	0.004	0.05	12.5	6.61	0.78	0.47	0.2	0.41	1.85	0.18	95.8	3.3	0.8	0.2
Ed.P 4	0.009	0.03	3.3	6.79	0.37	0.45	0.24	0.01	1.07	0.18	97.8	1.8	0.2	
Ky.I 1	0.209	6.37	30.5	7.3	0.55	0.42	0.69	0.54	4.91	14.2	75.4	12.2	11.7	0.7
Ky.I 2	0.227	6.44	28.4											
Pr.I 1	1.093	9.04	8.27	7.1	0.87	0.22	1.87	2.75	0.17	1.81	49.92	19	28.15	2.94
Pr.I 2	0.868	6.62	7.63							0.99				
Lk.F 1.1	0.019	0.33	17.4	7.81	2.12	0.45	1.15	2.06	3.53	0.18	87.2	3.2	6.3	3.3
Lk.F 1.2	0.01	0.03	3							0.473				
Lk.F 1.3	0.009	0.05	5.6							0.181				
Lk.H 1.1	0.008	0.11	13.8	7.98	0.07	0.36	0.88	0.84	2.16	0.088	93.4	2.7	3	1
Lk.H 1.2	0.0125	0.135	10.8							0.0895				
Lk.J 1.1	0.019	0.02	1.1	6.8	8.32	0.4	1.5	0.75	10.97	0.178	87.9	1.3	5.5	5.4
Lk.J 1.2	0.005	0.144	31.7	6.56	1.59	0.84	2.23	1.39	6.05	0.348	90.2	0.6	4.4	4.8

Table S2. Peak picking (chemical shift - ppm) and the corresponding peak intensities from ^{1}H-NMR spectra of extracts of Antarctic soils (Edmonson Point site 2) obtained by different extraction solvents: **A**) DMSO; **B**) H_2O/MeOH (1:1), and **C**) H_2O/MeCN (7:3). Samples were dissolved at final concentration of 15mg/mL in d_6-DMSO (**A**) and in 10 mM phosphate buffer (PB) in D_2O, pH 7.2 (**B** and **C**), with 0.5 mM TSP.

A) Extract in DMSO

	ppm	Intensity		ppm	Intensity
1	11.20	9587.7	21	2.61	66108.2
2	11.16	22425.4	22	2.57	11003.8
3	10.10	34779.8	23	2.55	3613277.1
4	9.21	13846.3	24	2.55	3445335.7
5	5.33	26736.5	25	2.51	1019956.1
6	5.18	14800.4	26	2.51	1049092.7
7	4.91	13703.4	27	2.43	26964.4
8	4.52	404886.9	28	2.39	18667.1
9	4.50	337998.6	29	2.34	78714.4
10	3.83	38107.4	30	2.34	76497.7
11	3.78	25219.8	31	2.02	29817.8
12	3.65	38621.7	32	1.97	24769.5
13	3.58	69298.2	33	1.46	18511.3
14	3.43	12643241.2	34	1.23	201231.9
15	3.25	67249.5	35	0.85	54955.9
16	3.11	42179.0	36	0.84	35204.9
17	2.99	19740.2	37	-0.02	108957.9
18	2.95	18507.1	38	-0.02	107391.9
19	2.89	15705.1	39	-0.07	50811.7
20	2.66	18724.4			

B) **Extract in H_2O/MeOH (1:1)**

	ppm	Intensity		ppm	Intensity		ppm	Intensity		ppm	Intensity		ppm	Intensity
1	8.46	51440.3	29	4.08	101194.0	57	3.69	351973.5	85	3.27	144877.1	113	2.05	53543.0
2	7.80	13443.0	30	4.07	124461.8	58	3.68	328503.4	86	3.26	149308.4	114	1.92	802985.4
3	7.43	15530.9	31	4.04	451400.3	59	3.67	334218.6	87	3.25	189643.8	115	1.91	66752.0
4	7.34	17883.8	32	4.02	464642.7	60	3.66	306297.0	88	3.23	128131.7	116	1.89	47364.0
5	6.91	21447.7	33	4.00	425342.1	61	3.65	140309.7	89	3.21	400527.3	117	1.55	63335.4
6	5.45	45882.4	34	3.97	148174.8	62	3.64	135520.7	90	3.13	189769.5	118	1.49	297026.2
7	5.41	57631.1	35	3.95	192246.7	63	3.63	89379.3	91	3.03	36154.3	119	1.48	294534.6

8	5.24	127509.9	36	3.94	207773.4	64	3.61	156905.6	92	3.02	54753.1	120	1.34	176357.0
9	5.23	131706.3	37	3.91	463480.6	65	3.59	354193.7	93	3.01	39728.7	121	1.32	200918.7
10	5.20	25506.0	38	3.91	422637.0	66	3.58	749761.9	94	2.81	21289.9	122	1.07	43908.2
11	5.03	49254.1	39	3.89	526814.2	67	3.57	390751.7	95	2.75	38972.7	123	1.06	70115.4
12	5.01	135206.8	40	3.89	677901.6	68	3.56	575194.0	96	2.73	189096.5	124	1.05	97251.0
13	4.66	81870.4	41	3.87	274009.6	69	3.54	194066.2	97	2.68	42406.9	125	1.04	98490.3
14	4.64	86334.9	42	3.85	289310.7	70	3.53	156981.9	98	2.66	25348.3	126	1.02	67044.7
15	4.29	49350.5	43	3.84	449947.4	71	3.53	179507.5	99	2.57	45428.6	127	1.01	64003.0
16	4.25	120277.1	44	3.82	424697.9	72	3.51	168317.9	100	2.54	35296.2	128	1.00	106806.6
17	4.24	110975.3	45	3.82	417394.4	73	3.49	288549.7	101	2.41	164889.4	129	0.99	107954.4
18	4.23	66451.2	46	3.81	715983.1	74	3.49	139011.5	102	2.39	36461.0	130	0.98	108624.2
19	4.22	68372.1	47	3.79	675202.1	75	3.48	232084.4	103	2.37	72169.0	131	0.97	172951.0
20	4.19	48404.4	48	3.78	391157.4	76	3.47	147863.4	104	2.36	71267.3	132	0.96	137115.7
21	4.17	69930.7	49	3.77	490907.1	77	3.46	135728.1	105	2.35	57081.1	133	0.94	97731.6
22	4.16	124680.0	50	3.76	498605.7	78	3.45	212614.2	106	2.31	52425.1	134	0.93	73225.6
23	4.15	155020.9	51	3.75	439533.4	79	3.44	190093.4	107	2.30	88457.7	135	0.91	68201.7
24	4.13	222473.2	52	3.74	434494.2	80	3.43	123390.1	108	2.29	60318.8	136	0.88	92735.8
25	4.13	170962.3	53	3.73	421290.7	81	3.42	245813.2	109	2.19	65053.3	137	0.07	134037.2
26	4.12	275922.0	54	3.72	925612.0	82	3.41	140702.8	110	2.17	81205.8	138	-0.00	1438756.8
27	4.11	303613.2	55	3.71	345560.0	83	3.40	205646.8	111	2.08	50906.1			
28	4.10	87891.3	56	3.70	835031.3	84	3.39	82366.6	112	2.06	59509.8			

C) Extract in H_2O/MeCN (7:3)

	ppm	Intensity		ppm	Intensity		ppm	Intensity		ppm	Intensity		ppm	Intensity		ppm	Intensity		ppm	Intensity
1	8.46	50610.4	29	4.27	81580.0	57	3.96	136573.4	85	3.67	384513.6	113	3.41	231291.4	141	2.41	203660.0	169	1.55	55835.5
2	7.81	20547.2	30	4.26	82106.1	58	3.95	191953.8	86	3.67	277847.3	114	3.40	75389.3	142	2.39	31101.6	170	1.49	346385.9
3	7.80	20422.3	31	4.25	125974.1	59	3.94	224191.1	87	3.66	340688.1	115	3.39	97516.4	143	2.37	74812.1	171	1.48	342652.7
4	7.43	15497.9	32	4.24	111560.9	60	3.93	189906.8	88	3.65	154324.8	116	3.35	16337.6	144	2.37	47477.1	172	1.37	44376.5
5	7.42	11907.9	33	4.23	77851.5	61	3.91	549630.3	89	3.64	128489.5	117	3.30	22380.5	145	2.36	73448.9	173	1.34	405440.9
6	7.34	18307.5	34	4.22	85490.3	62	3.91	465256.0	90	3.63	92222.0	118	3.28	33721.2	146	2.35	61810.7	174	1.33	428832.5
7	7.33	15700.5	35	4.20	47201.1	63	3.89	597411.9	91	3.61	170097.2	119	3.27	161270.5	147	2.31	66142.3	175	1.21	30153.9
8	7.19	11244.9	36	4.19	63560.7	64	3.89	733553.3	92	3.59	369957.4	120	3.26	176459.5	148	2.30	122110.8	176	1.20	27184.2
9	6.93	18482.9	37	4.17	77493.4	65	3.87	275825.5	93	3.58	895058.5	121	3.25	213942.4	149	2.29	74961.8	177	1.07	43796.0
10	6.91	25462.6	38	4.16	132448.5	66	3.86	277907.9	94	3.57	432328.8	122	3.23	155039.5	150	2.24	75184.2	178	1.06	72256.4
11	6.52	9541.9	39	4.15	160190.9	67	3.85	306933.9	95	3.56	674250.1	123	3.21	372034.2	151	2.19	66217.4	179	1.05	106281.0

12	5.91	9738.1	40	4.13	267728.6	68	3.84	465836.6	96	3.55	205203.9	124	3.19	21846.8	152	2.18	83267.5	180	1.05	48297.4
13	5.45	44108.1	41	4.13	232391.6	69	3.82	452169.8	97	3.55	205012.4	125	3.13	109194.0	153	2.16	57683.3	181	1.04	106006.3
14	5.42	57789.1	42	4.12	310111.4	70	3.82	435664.8	98	3.54	189750.8	126	3.11	24146.2	154	2.14	45863.9	182	1.02	71298.9
15	5.25	23676.8	43	4.11	345768.0	71	3.81	863996.1	99	3.53	158109.2	127	3.03	57632.1	155	2.13	43335.7	183	1.01	67222.6
16	5.24	153725.0	44	4.11	122158.4	72	3.79	809133.3	100	3.53	195127.3	128	3.02	75479.8	156	2.12	42168.0	184	1.00	118431.7
17	5.23	152981.2	45	4.10	97903.6	73	3.78	471955.9	101	3.51	197941.6	129	3.01	54298.3	157	2.08	62065.7	185	0.99	121299.8
18	5.20	32191.9	46	4.08	115918.2	74	3.77	552899.9	102	3.49	342666.7	130	2.84	16278.7	158	2.06	61584.4	186	0.98	116099.0
19	5.20	32220.9	47	4.07	135061.1	75	3.76	527360.1	103	3.49	135528.5	131	2.81	23136.1	159	2.05	53461.4	187	0.97	190080.3
20	5.04	50035.5	48	4.06	109259.5	76	3.75	443997.1	104	3.48	256794.5	132	2.80	23905.1	160	2.02	31940.8	188	0.96	143820.0
21	5.03	53339.3	49	4.04	485924.1	77	3.74	454134.6	105	3.47	141423.1	133	2.75	42392.8	161	2.01	37817.9	189	0.94	100433.4
22	5.02	113810.2	50	4.02	487871.9	78	3.73	444241.0	106	3.47	147047.2	134	2.72	21892.3	162	1.92	1223068.9	190	0.93	69857.4
23	5.01	146966.8	51	4.01	128996.2	79	3.72	1116757.7	107	3.47	146219.8	135	2.70	34761.0	163	1.91	80859.6	191	0.92	57219.5
24	4.97	17817.8	52	4.00	426655.4	80	3.71	376082.6	108	3.46	220904.7	136	2.68	32339.5	164	1.89	55852.9	192	0.91	54953.9
25	4.66	12541.4	53	4.00	460894.8	81	3.70	1076639.0	109	3.44	183741.4	137	2.67	25535.3	165	1.88	30784.3	193	0.88	77458.6
26	4.64	17326.3	54	4.00	418471.9	82	3.70	524108.2	110	3.43	132919.7	138	2.65	22107.6	166	1.73	32429.5	194	-0.00	1125706.5
27	4.30	50839.9	55	3.98	123196.6	83	3.69	362244.0	111	3.42	290503.4	139	2.57	23195.8	167	1.72	31875.4			
28	4.29	51880.6	56	3.97	148853.1	84	3.68	360650.4	112	3.41	148886.5	140	2.44	23971.8	168	1.70	31870.5			

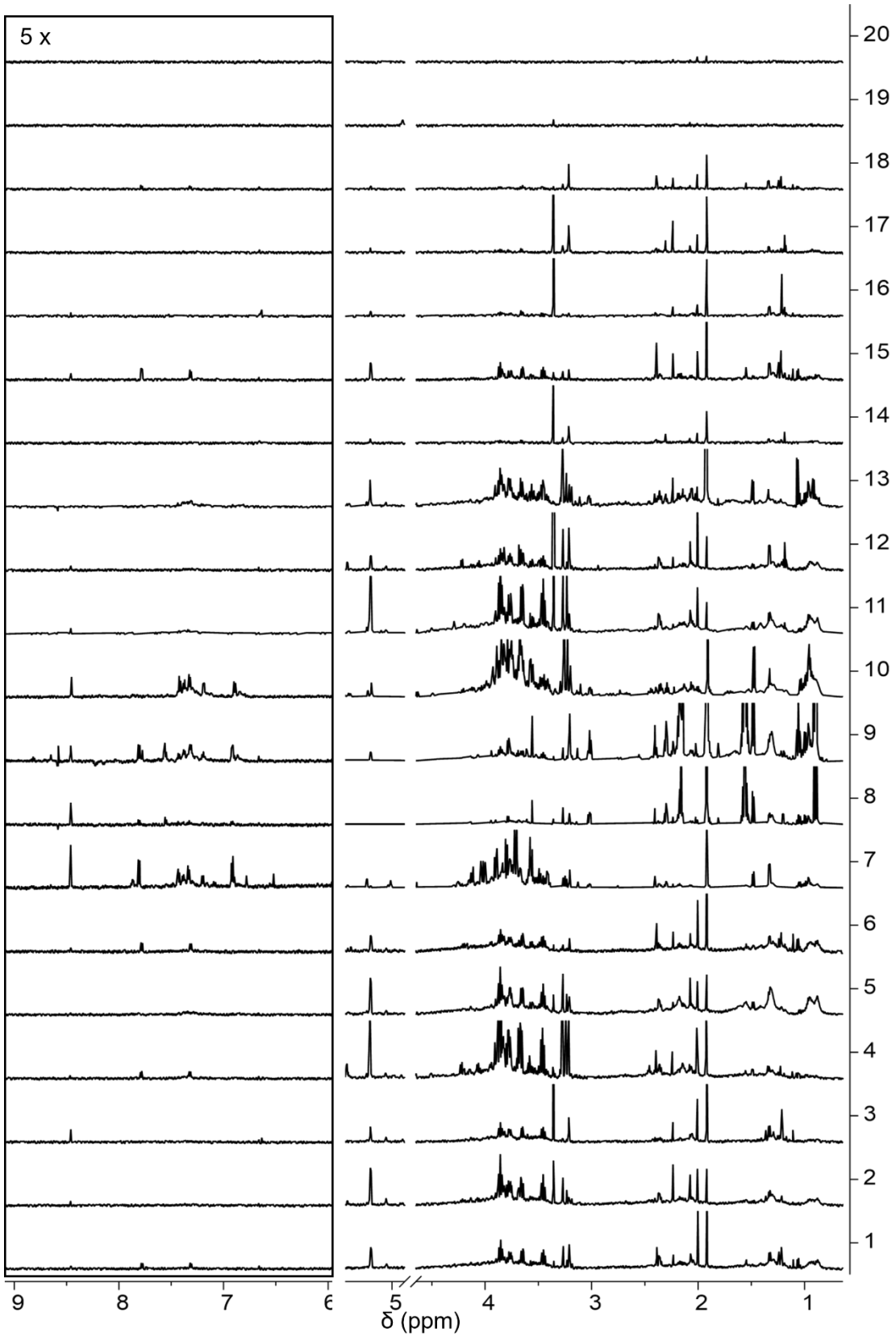


Figure S1. [1]H-NMR spectra of extracts of Antarctic soils from different sites obtained in H_2O/MeCN (7:3). Samples are dissolved at final concentration of 15mg/mL in 10 mM PB in D_2O, pH 7.2, with 0.5 mM TSP. 1) Ap.I 1; 2) Ap.I 2; 3) B.By 1; 4) B.By 2; 5) Cp.K 1; 6)Cp.K 2; 7) Ed.P 2; 8) Ed.P 3; 9) Ed.P 4; 10) Ky.I 1; 11) Ky.I 2; 12) Pr.I 1; 13) Pr.I 2; 14) Lk.F 1.1; 15) Lk.F 1.2; 16) Lk.F 1.3; 17) Lk.H 1.1; 18) Lk.H 1.2; 19) Lk.J 1.1; 20) Lk.J 1.2.

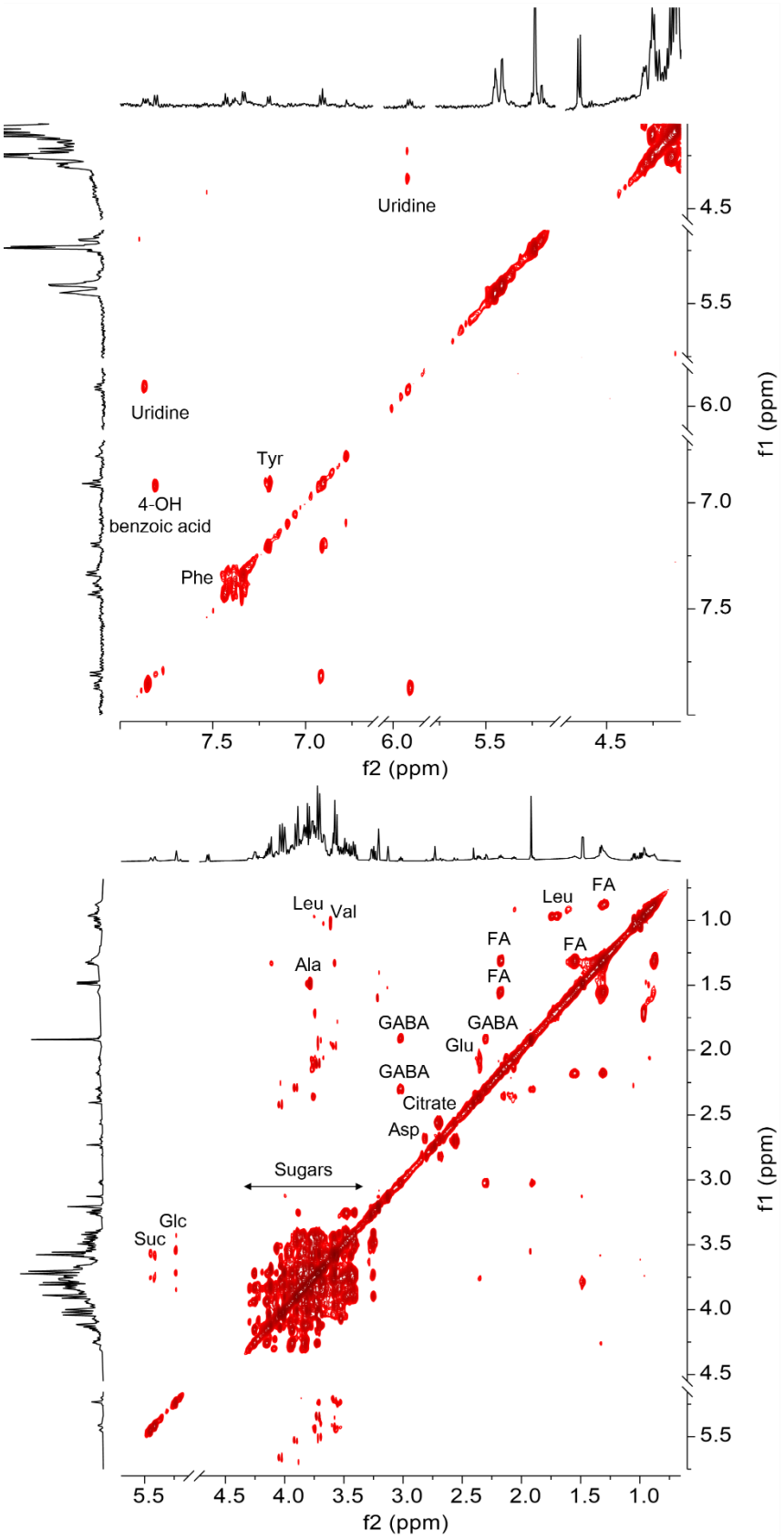


Figure S2. Expansions of two regions of $^{1}H,^{1}H$-TOCSY spectrum of Antarctic soil extract Ed.P 2 in 10 mM PB in D_2O. The corresponding ^{1}H-NMR spectra are reported along the axes. The resonances of some of the most important metabolites are indicated on the 2D spectra.

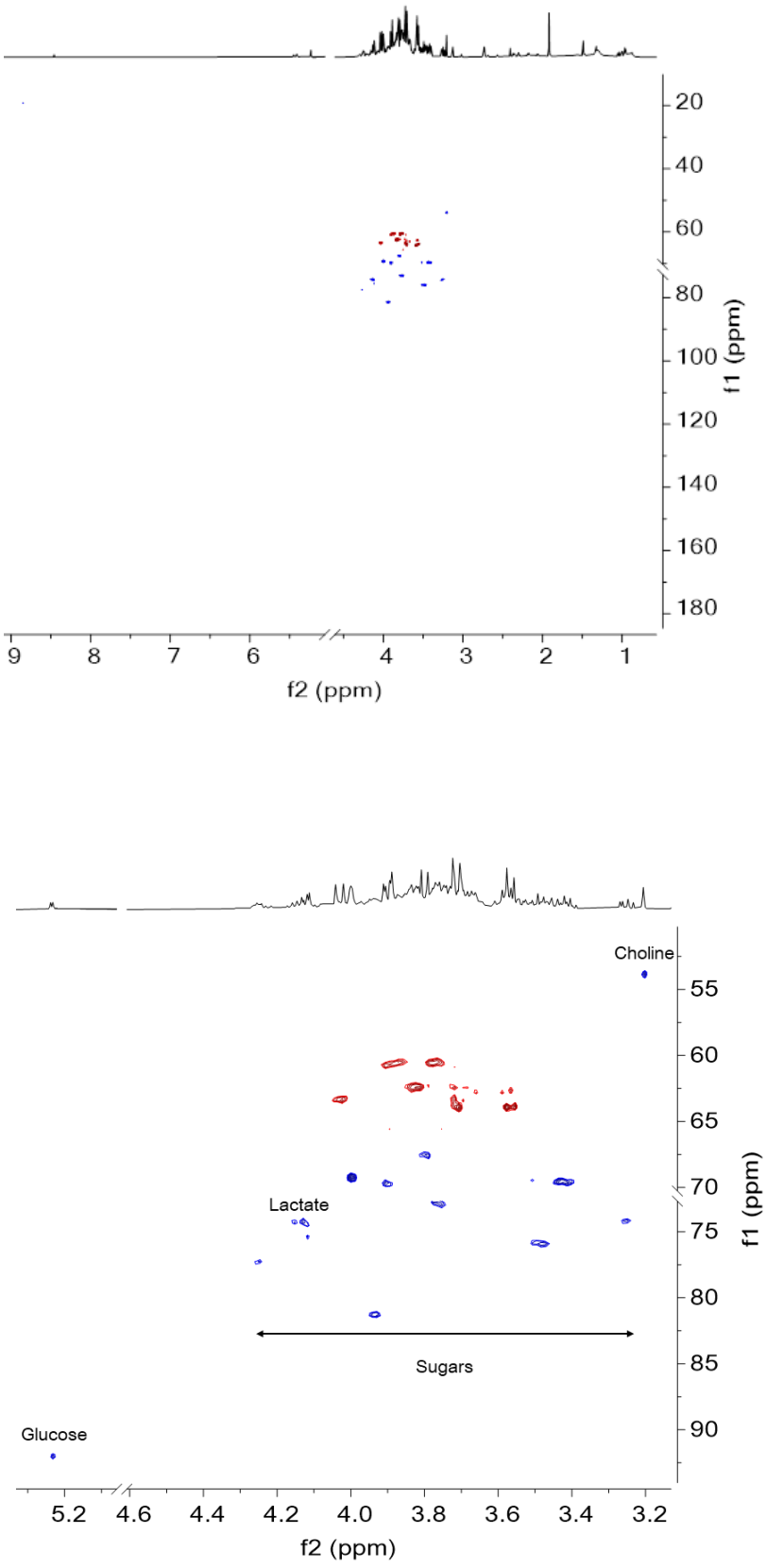


Figure S3. $^{1}H,^{13}C$-HSQC spectrum of Antarctic soil extract Ed.P 2 in 10 mM PB in D_2O. The corresponding ^{1}H-NMR spectrum is reported.

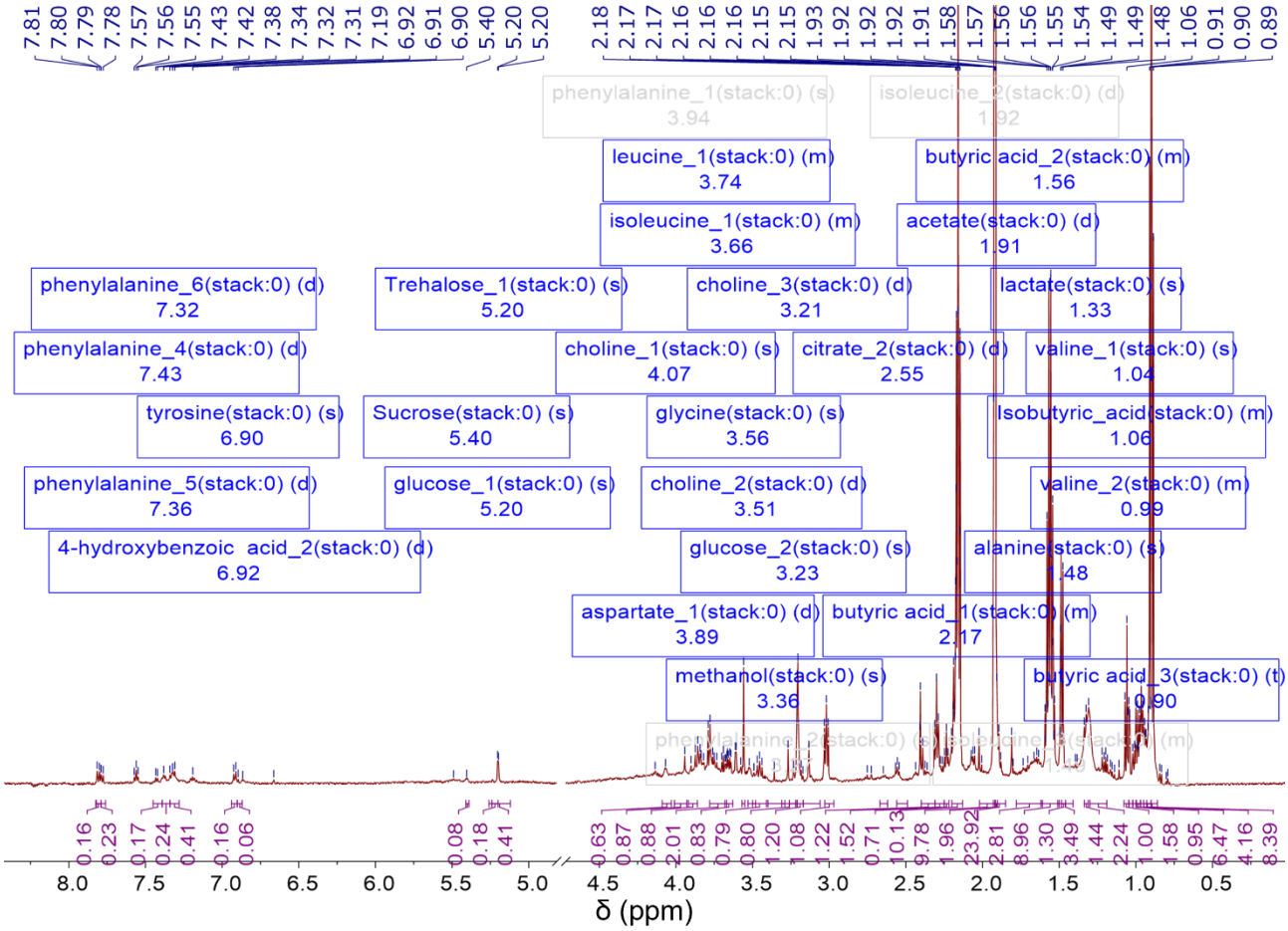
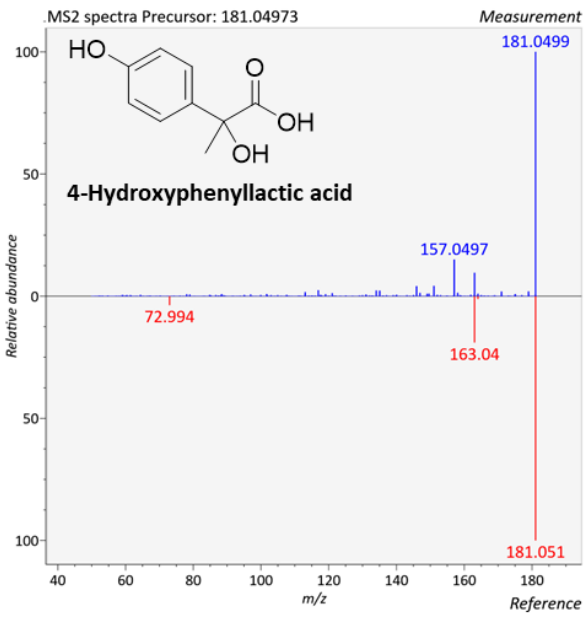
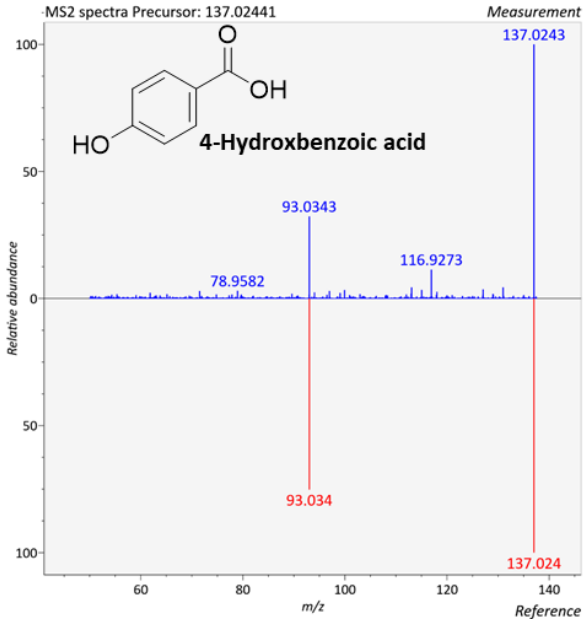


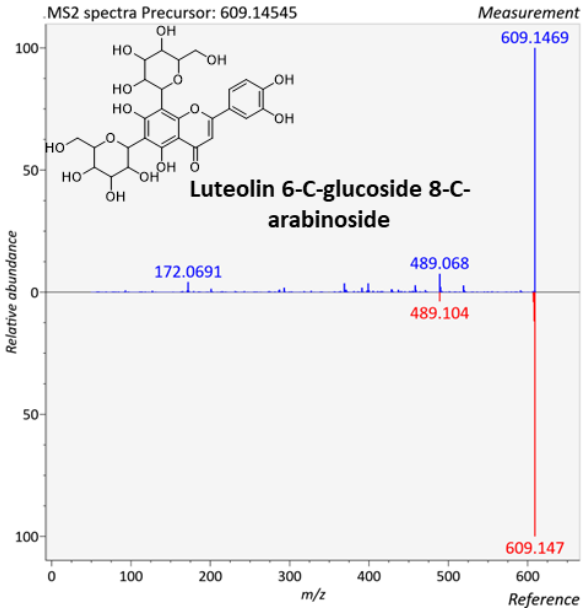
Figure S4. Output of SMA plug-in of MestreNova software on a spectrum of Antarctic soil extract Ed.P 4 in 10 mM PB in D_2O. Labels with the name of the molecule, the chemical shift and multiplicity are automatically reported for each signal.

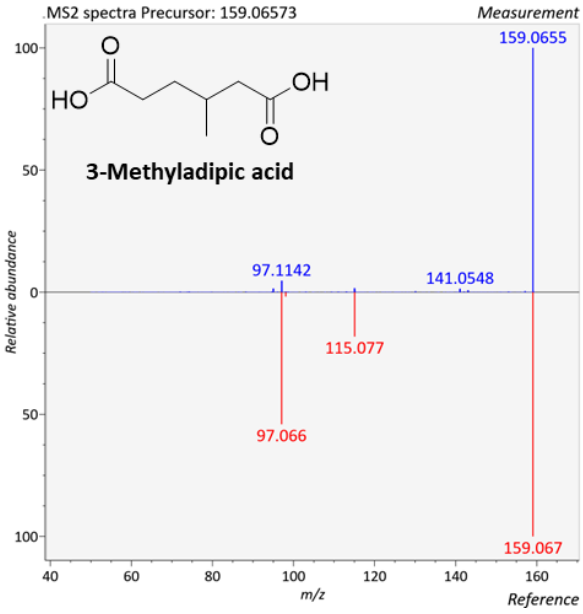
MS/MS spectra of identified compounds

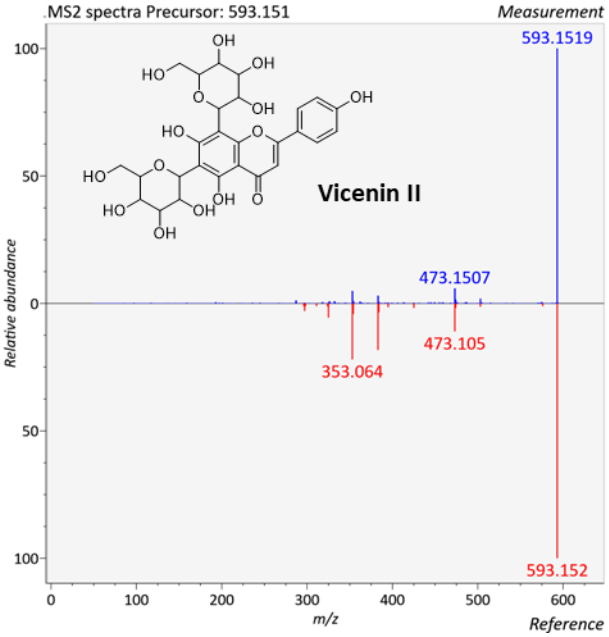
Experimental spectra (ES-) reported in comparison with matched reference spectra.







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Measurement
593.1519
Vicenin II
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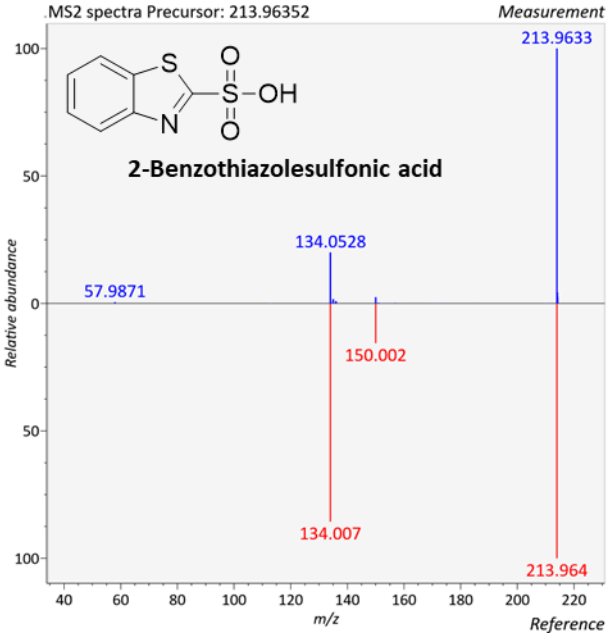
473.105
353.064
593.152
Relative abundance
m/z
Reference


MS2 spectra Precursor: 213.96352
Measurement
213.9633
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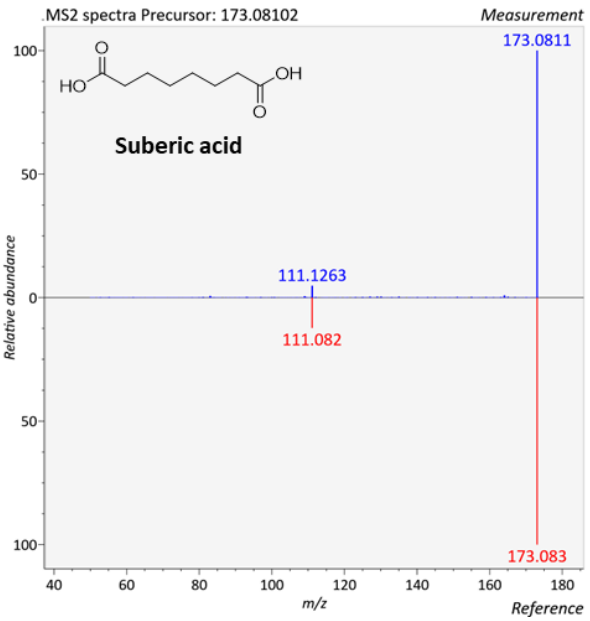
57.9871
150.002
134.007
213.964
Relative abundance
m/z
Reference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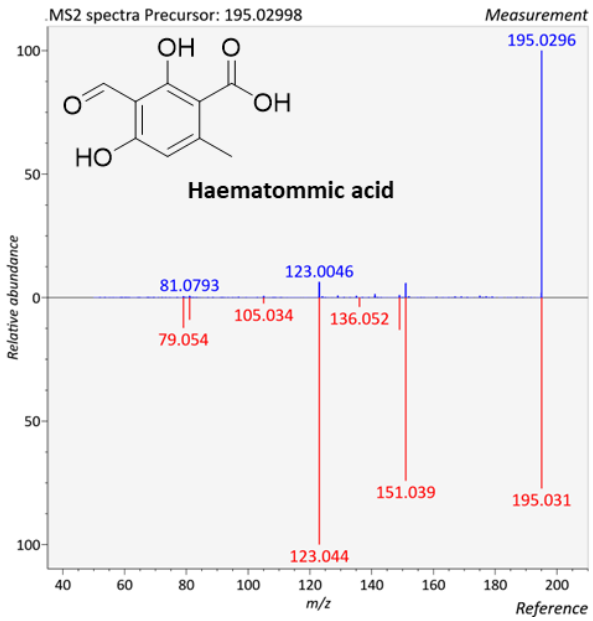
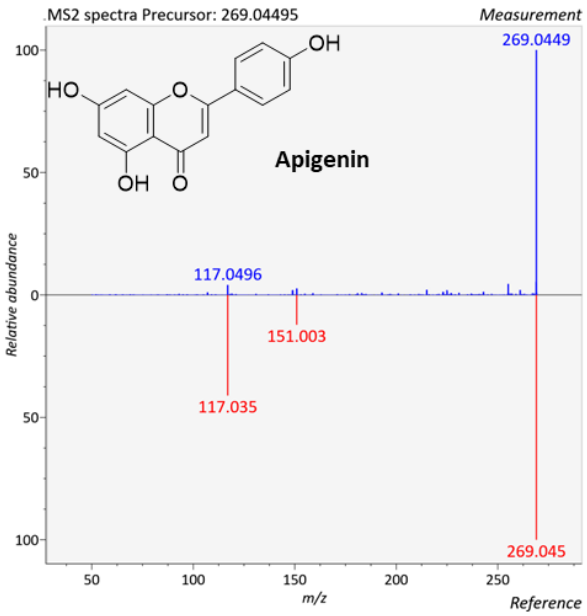
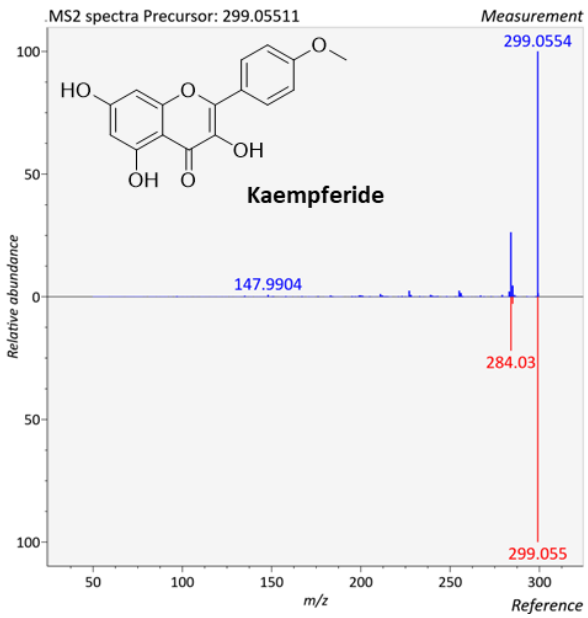
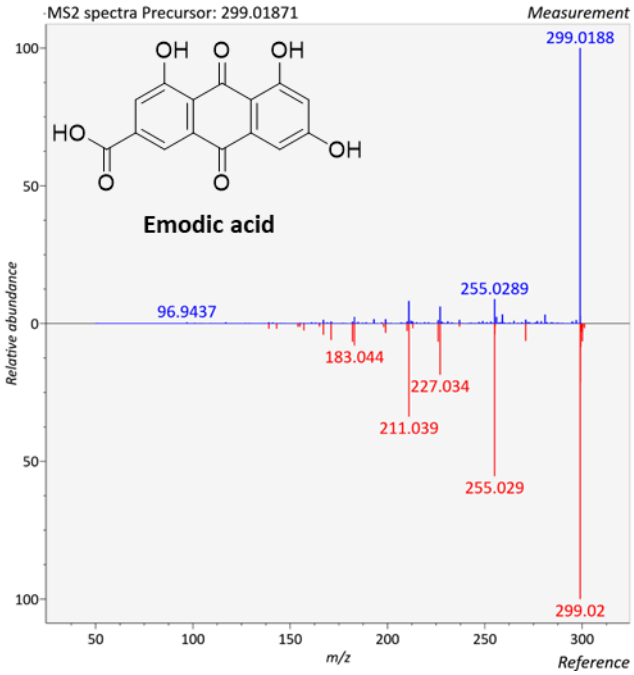
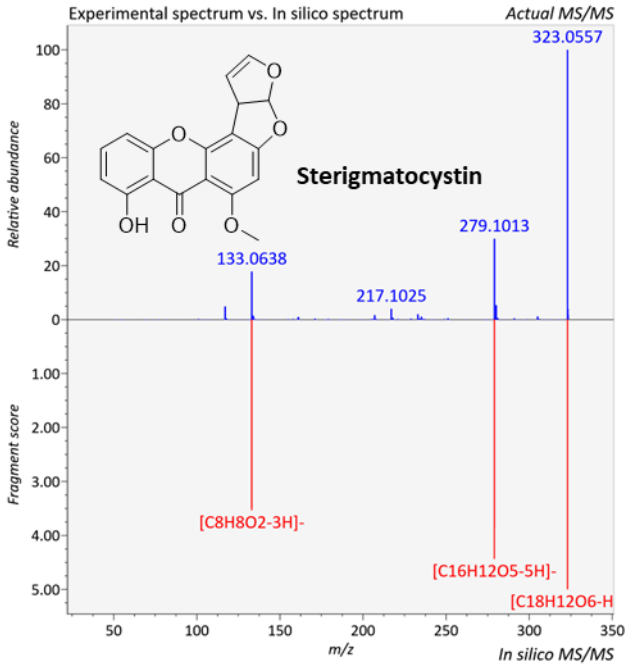
Measurement
173.0811
Suberic acid
111.1263
111.082
173.083
Relative abundance
m/z
Reference

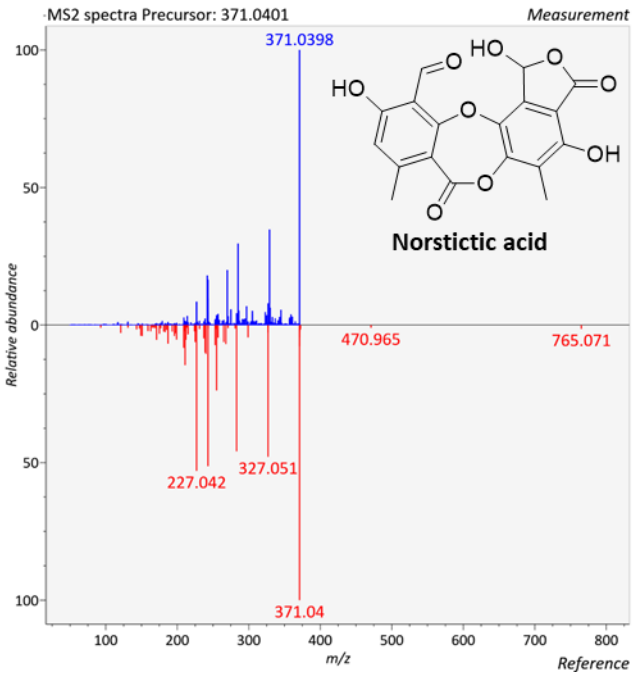

MS2 spectra Precursor: 195.02998
Measurement
195.0296
Haematommic acid
123.0046
81.0793
105.034
136.052
79.054
151.039
195.031
123.044
Relative abundance
m/z
Reference

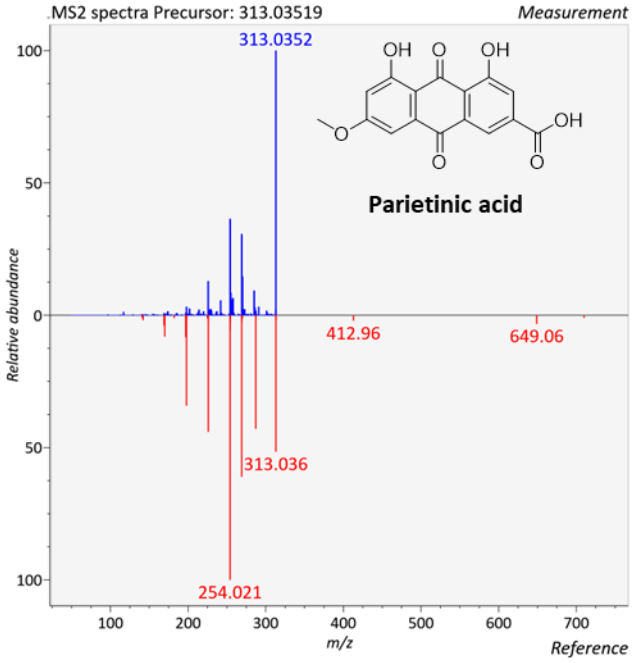

MS2 spectra Precursor: 269.04495
Measurement
269.0449
Apigenin
117.0496
151.003
117.035
269.045
Relative abundance
m/z
Reference

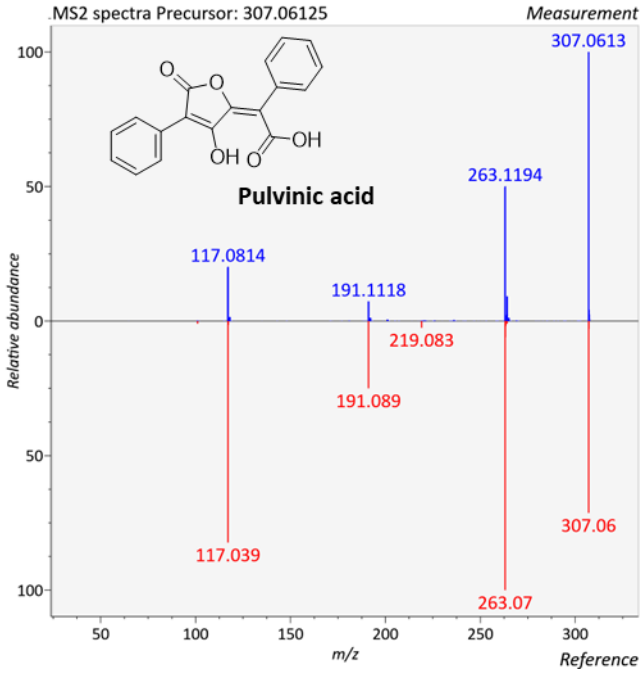

MS2 spectra Precursor: 299.05511
Measurement
299.0554
Kaempferide
147.9904
284.03
299.055
Relative abundance
m/z
Reference

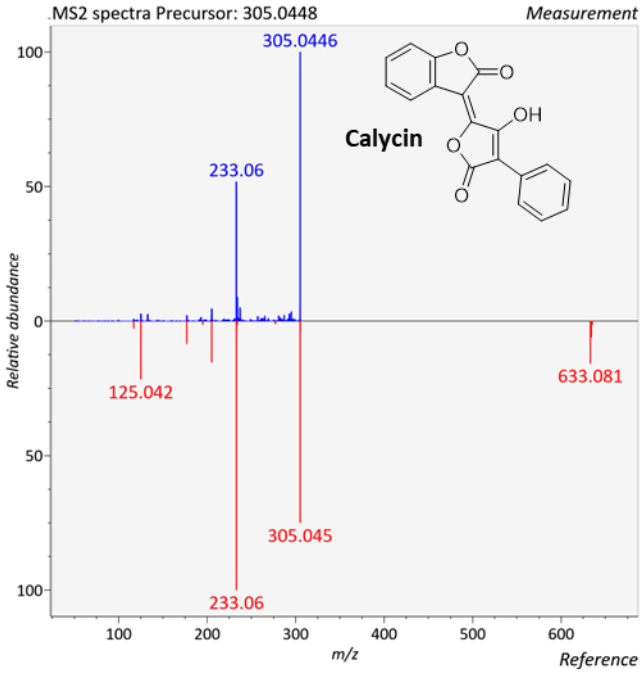

MS2 spectra Precursor: 299.01871
Measurement
299.0188
Emodic acid
255.0289
96.9437
183.044
227.034
211.039
255.029
299.02
Relative abundance
m/z
Reference

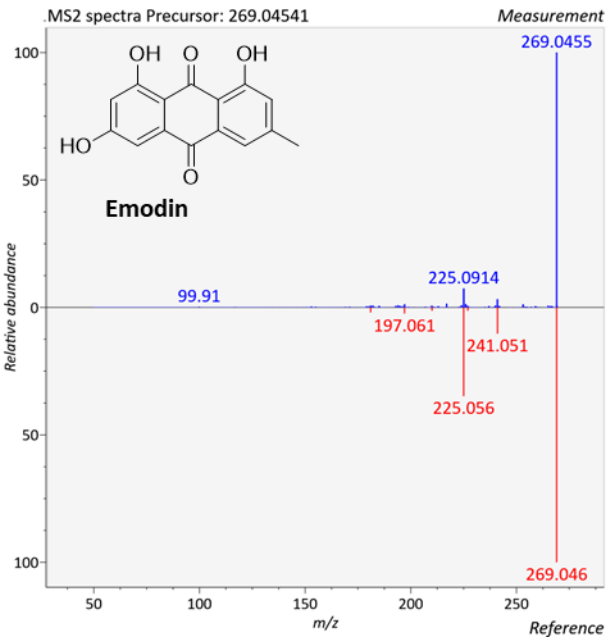

Experimental spectrum vs. In silico spectrum
Actual MS/MS
323.0557
Sterigmatocystin
279.1013
133.0638
217.1025
[C8H8O2-3H]-
[C16H12O5-5H]-
[C18H12O6-H
Relative abundance
Fragment score
m/z
In silico MS/MS

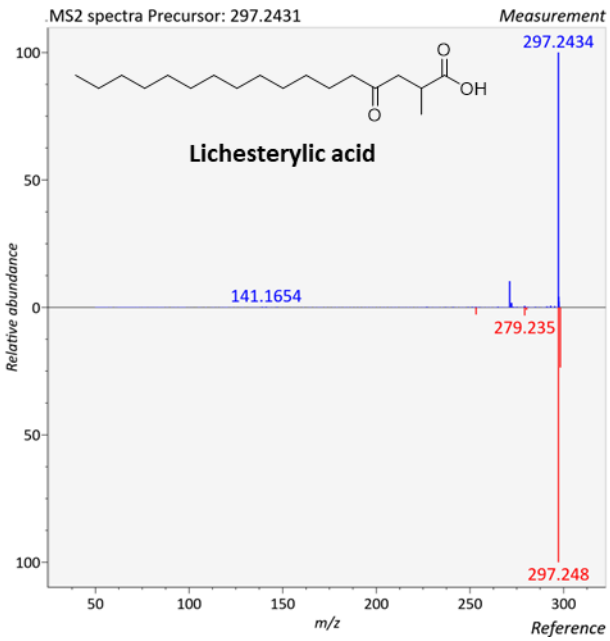

MS2 spectra Precursor: 371.0401
Measurement
371.0398
Norstictic acid
470.965
765.071
227.042
327.051
371.04
Relative abundance
m/z
Reference

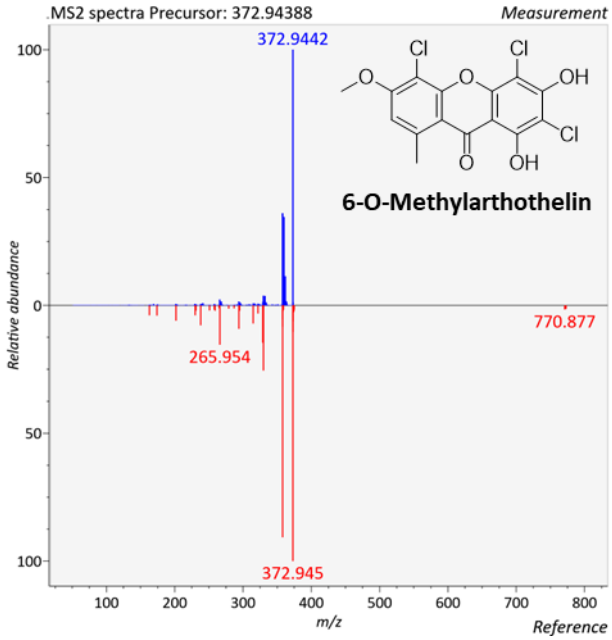

MS2 spectra Precursor: 313.03519
Measurement
313.0352
Parietinic acid
412.96
649.06
313.036
254.021
Relative abundance
m/z
Reference


MS2 spectra Precursor: 307.06125
Measurement
307.0613
Pulvinic acid
263.1194
117.0814
191.1118
219.083
191.089
307.06
117.039
263.07
Relative abundance
m/z
Reference

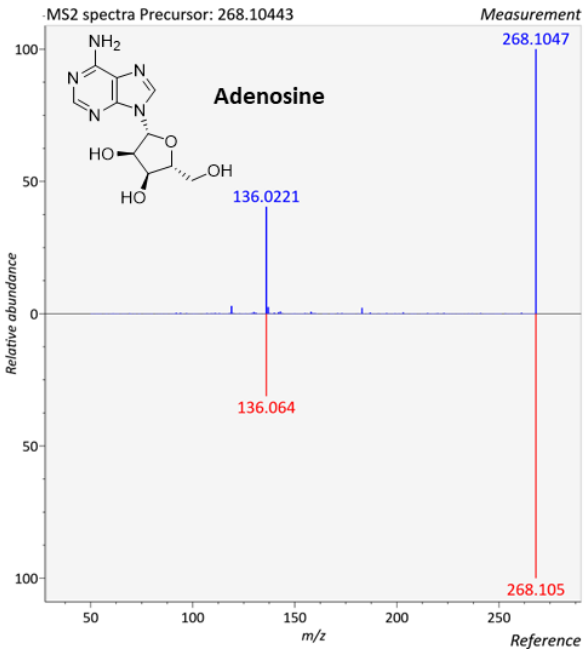

MS2 spectra Precursor: 305.0448
Measurement
305.0446
Calycin
233.06
633.081
125.042
305.045
233.06
Relative abundance
m/z
Reference

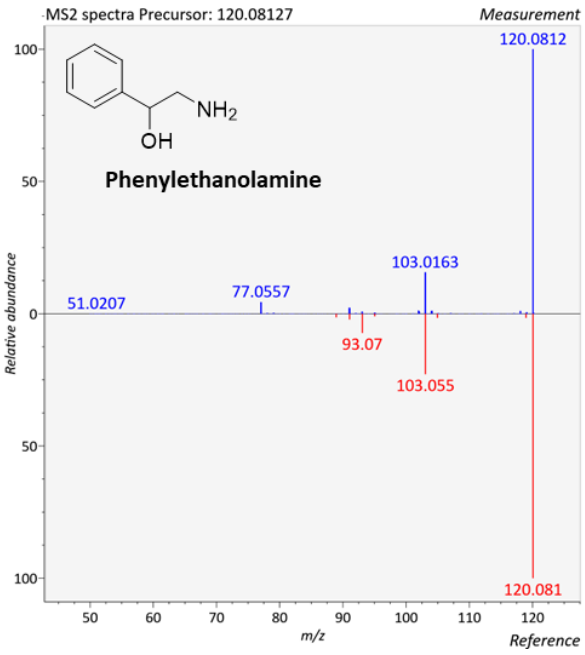

MS2 spectra Precursor: 269.04541
Measurement
269.0455
OH
O
OH
HO
O
Emodin
225.0914
99.91
197.061
241.051
225.056
269.046
Relative abundance
m/z
Reference

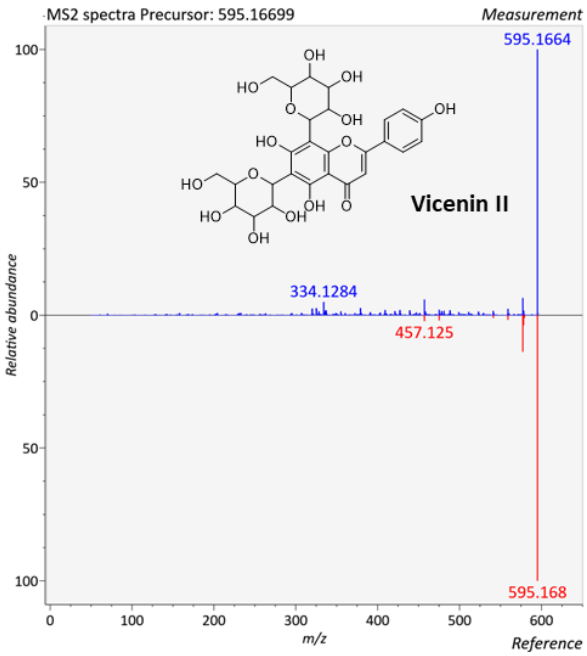

MS2 spectra Precursor: 297.2431
Measurement
297.2434
O
OH
O
Lichesterylic acid
141.1654
279.235
297.248
Relative abundance
m/z
Reference

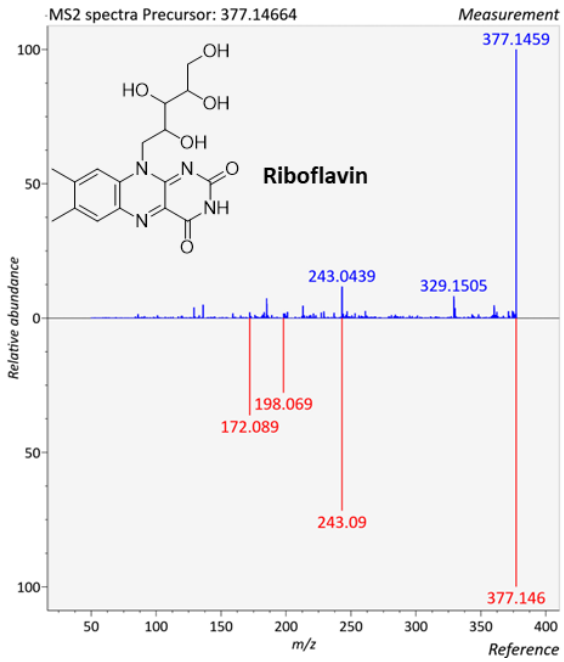

MS2 spectra Precursor: 372.94388
Measurement
372.9442
Cl
Cl
O
O
OH
Cl
O
OH
6-O-Methylarthothelin
770.877
265.954
372.945
Relative abundance
m/z
Reference

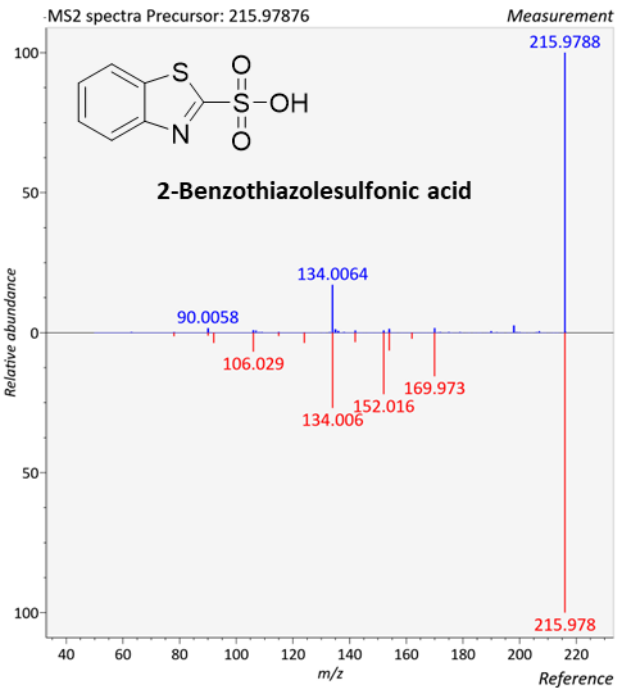
Experimental spectra (ES+) reported in comparison with matched reference spectra.

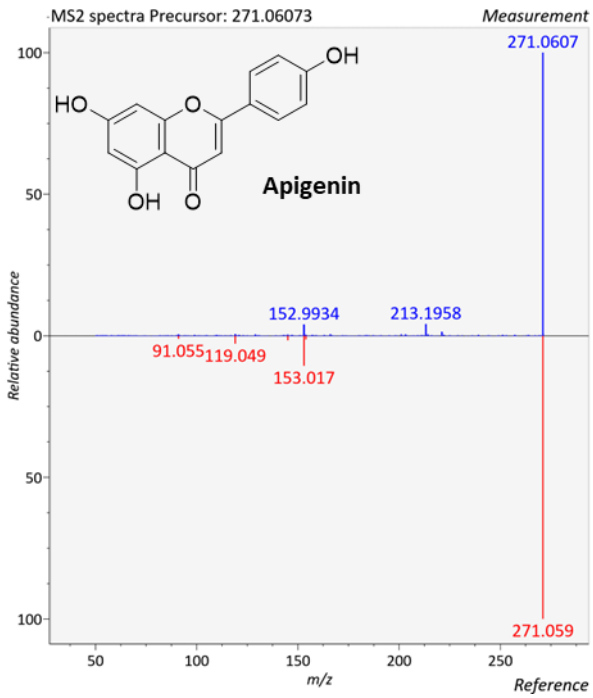


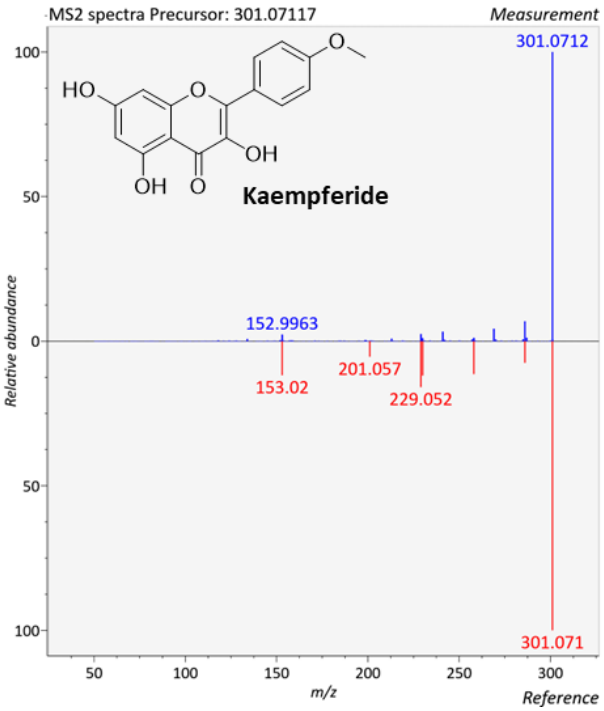


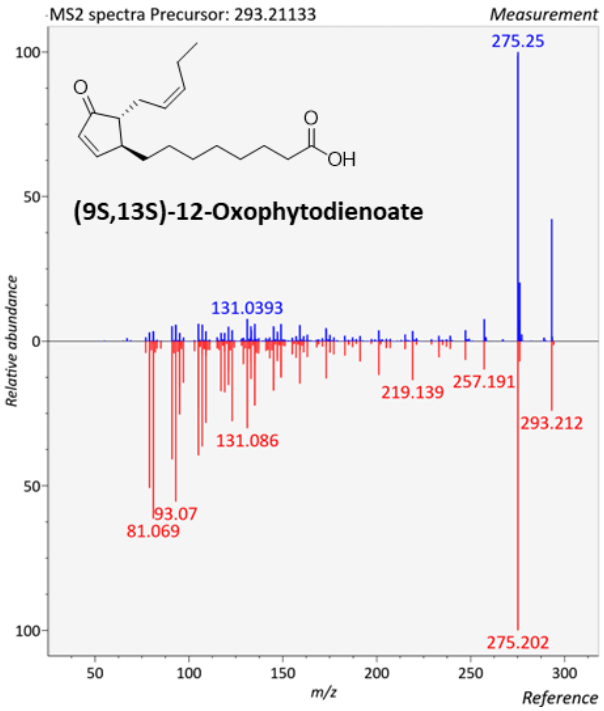


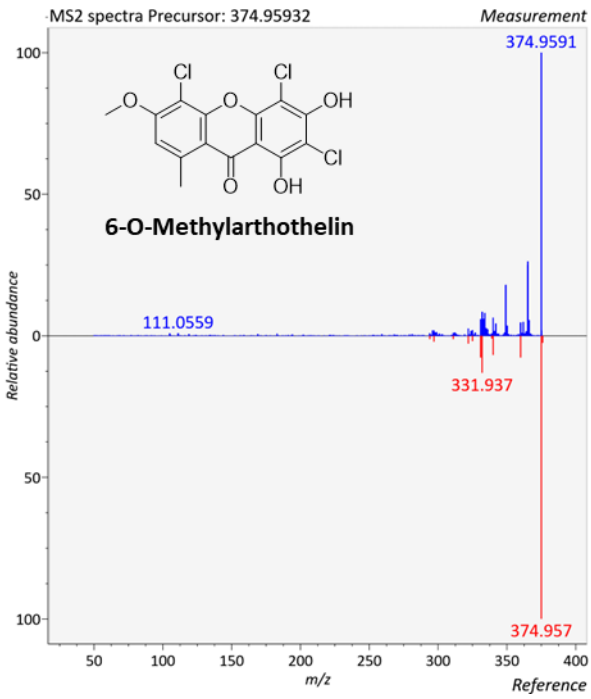



MS2 spectra Precursor: 215.97876
Measurement
215.9788
2-Benzothiazolesulfonic acid
134.0064
90.0058
106.029
134.006
152.016
169.973
215.978
Relative abundance
m/z
Reference


MS2 spectra Precursor: 271.06073
Measurement
271.0607
Apigenin
152.9934
213.1958
91.055
119.049
153.017
271.059
Relative abundance
m/z
Reference


MS2 spectra Precursor: 301.07117
Measurement
301.0712
Kaempferide
152.9963
153.02
201.057
229.052
301.071
Relative abundance
m/z
Reference


MS2 spectra Precursor: 293.21133
Measurement
275.25
(9S,13S)-12-Oxophytodienoate
131.0393
219.139
257.191
293.212
131.086
93.07
81.069
275.202
Relative abundance
m/z
Reference


MS2 spectra Precursor: 374.95932
Measurement
374.9591
Cl
Cl
O
O
OH
Cl
O
OH
6-O-Methylarthothelin
111.0559
331.937
374.957
Relative abundance
100
50
0
50
100
50
100
150
200
250
300
350
400
m/z
Reference