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Supplementary data

Structural characterization and anti-osteoporosis activity of two polysaccharides from the rhizome of *Curculigo orchioides*

Supplementary Tables

Table S1

The elution program of HPLC.

Time (min)	A%	B%	C%
0	0	17	83
24	0	17	83
25	0	19	81
30	0	18	82
31	5	14	81
32	5	14	81
33	0	18	82
34	5	14	81
36	0	18	82

A: ultrapure water

B: acetonitrile

C: 0.05 M phosphate buffer solution (pH 6.7)

Table S2

Static parameters of proximal tibia metaphysis.

Group	Tb.Ar %	Tb.Th *μ*m	Tb.N mm	Tb.Sp *μ*m
Sham	25.43±1.09	33775.52±1672.69	7.53±0.19	99042.21±2892.40
OVX	6.62±0.03##	33744.45±968.78	1.96±0.06##	475835.55±14803.72##
E2	16.71±0.13**	39008.64±1343.73**	4.29±0.17**	194433.24±7513.15**
CO50	16.94±0.65**	42674.52±483.83**	3.97±0.18**	209520.56±10915.22**

Values are expressed as mean ± SD. ## $P < 0.01$ *vs.* Sham; ** $P < 0.01$ *vs.* OVX.

Table S3

GC-MS analysis of COP50-1.

t_R (min)	PMAA	Type of linkage	Relative Molar radio	Mass fragments (m/z)
15.76	1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol	D-Glc*p*-(1→	4.5	43,59,71,87,101,117,129,145,161,205
16.21	1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-galactitol	D-Gal*p*-(1→	2.2	43,59,71,87,101,117,129,145,161,205
18.34	1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methyl-D-glucitol	→4)-D-Glc*p*-(1→	6.1	43,57,71,87,99,117,129,143,159,173,187,203,233
19.24	1,3,4,5-tetra-*O*-acetyl-2,6-di-*O*-methyl-D-glucitol	→3,4)-D-Glc*p*-(1→	2.2	43,59,75,87,117,129,143,161,185,203,233,305
20.05	1,4,5,6-tetra-*O*-acetyl-2,3-di-*O*-methyl-D-glucitol	→4,6)-D-Glc*p*-(1→	2.1	43,57,75,85,101,117,127,143,171,187,201,231,261
20.18	1,4,5,6-tetra-*O*-acetyl-2,3-di-*O*-methyl-D-mannitol	→4,6)-D-Man*p*-(1→	1.0	43,57,75,85,101,117,127,141,171,187,201,231,261

Table S4

^{1}H and ^{13}C NMR chemical shifts of COP50-1.

Glycosyl residue	**H1**	**H2**	**H3**	**H4**	**H5**	**H6**
	C1	**C2**	**C3**	**C4**	**C5**	**C6**
→4)-*α*-D-Glc*p*-(1→	5.33	3.78	3.66	3.77	3.53	3.55/3.57
Residue A	99.5	72.8	75.1	76.9	73.7	60.7
α-D-Glc*p*-(1→	5.32	3.76	3.69	3.55	3.57	3.52/3.53
Residue B	96.6	71.7	75.9	71.4	73.1	60.3
→3,4)-*α*-D-Glc*p*-(1→	4.57	3.70	4.07	3.72	3.59	3.59/3.60
Residue D	95.7	72.9	78.8	76.1	73.0	62.5
→4,6)-*α*-D-Glc*p*-(1→	4.90	3.77	3.65	3.71	3.61	3.76/3.78
Residue E	98.3	73.2	76.1	77.3	72.7	69.3
β-D-Gal*p*-(1→	4.56	3.88	3.56	3.71	3.67	3.34/3.36
Residue F	104.2	72.8	74.2	71.1	74.5	60.9
→4,6)-*β*-D-Man*p*-(1→	4.45	3.68	3.73	3.86	3.64	3.69/3.70
Residue G	102.6	72.9	73.4	76.0	74.5	69.4

Supplementary Figs

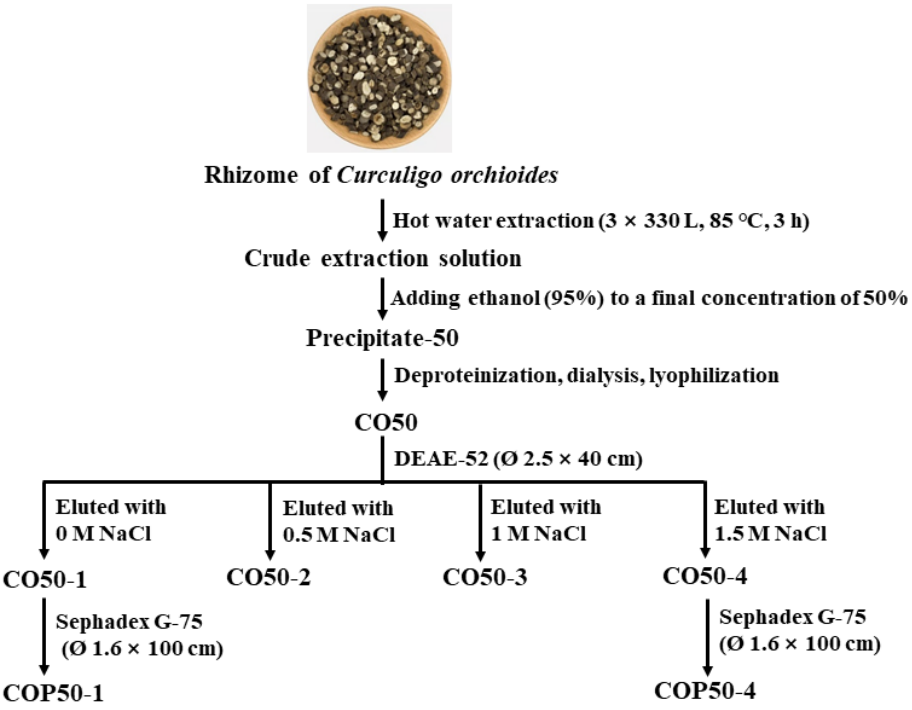


Fig. S1 The flow diagram for the isolation and purification of polysaccharides from *Curculigo orchioides*.

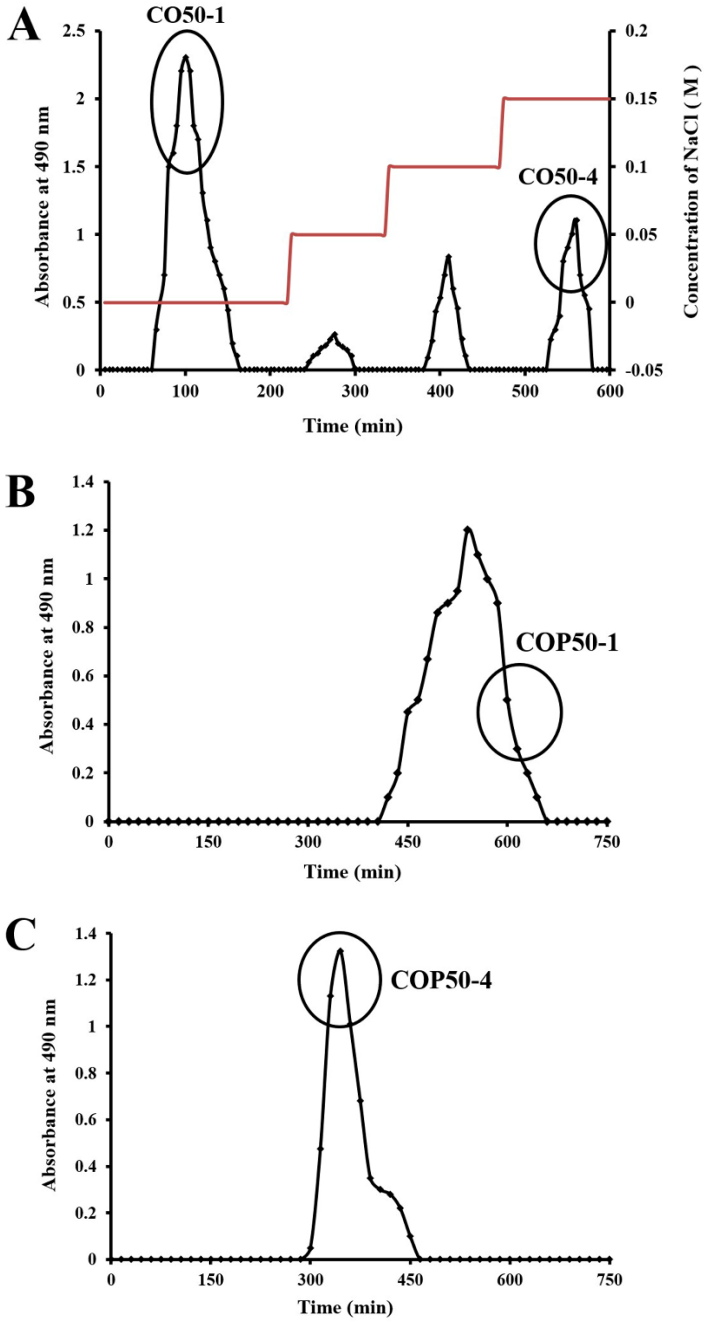


Fig. S2 (A) Elution profile of CO50 on DEAE-cellulose 52 column. Elution profile of CO50-1(B) and CO50-4 (C) on Sephadex G-75 column.

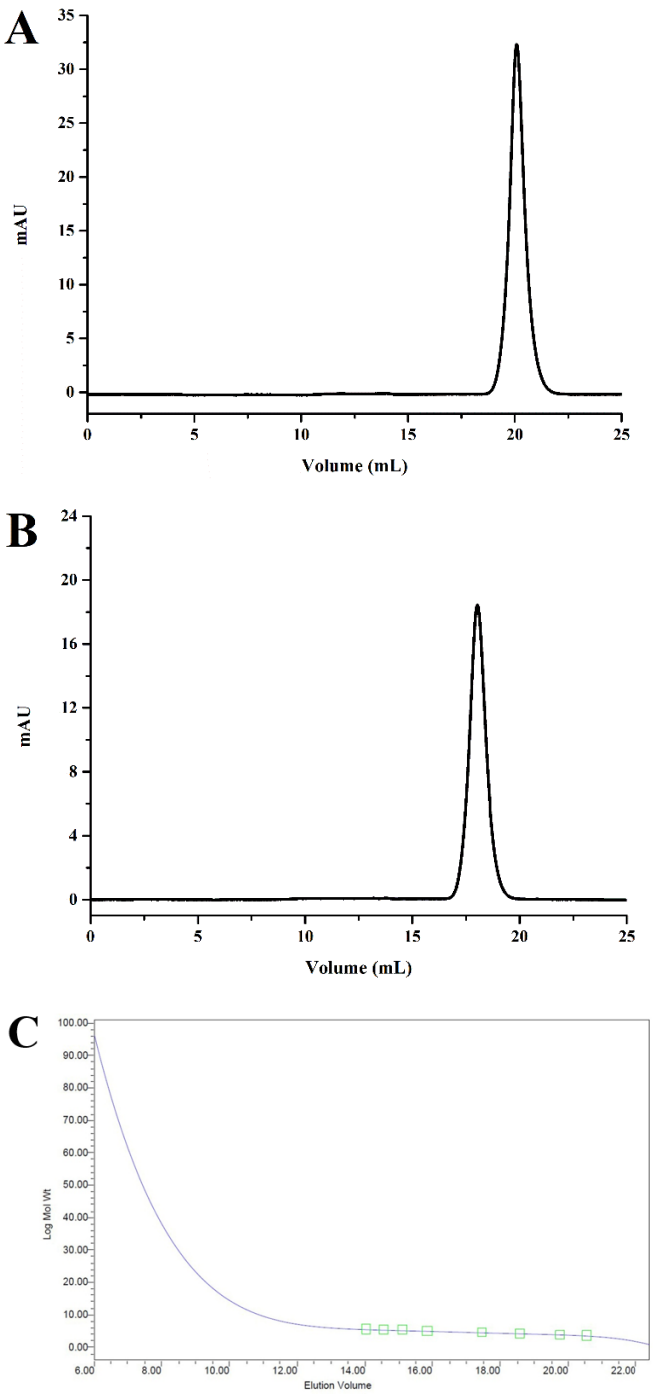


Fig. S3 The HPGPC chromatogram of COP50-1 (A) and COP50-4 (B). (C) The calibration curve of HPGPC.

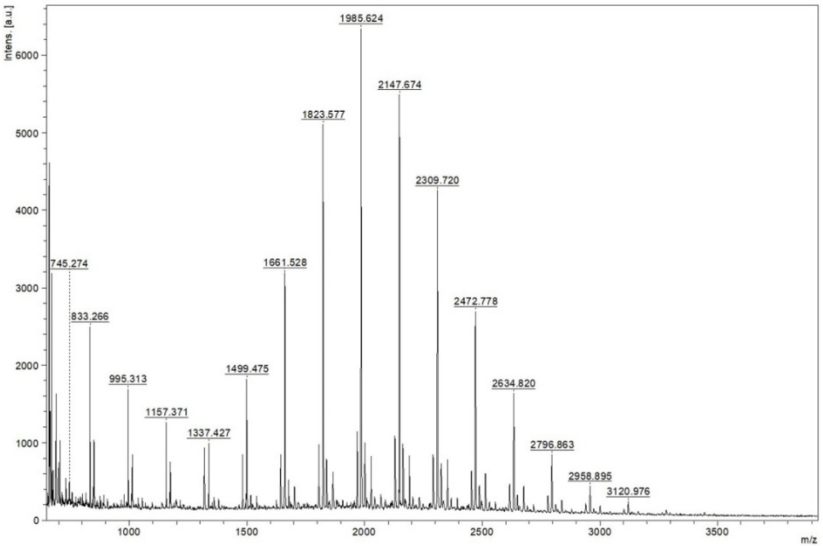


Fig. S4 The MALDI-TOF-MS spectrum of COP50-1 in 500-4000 Da.

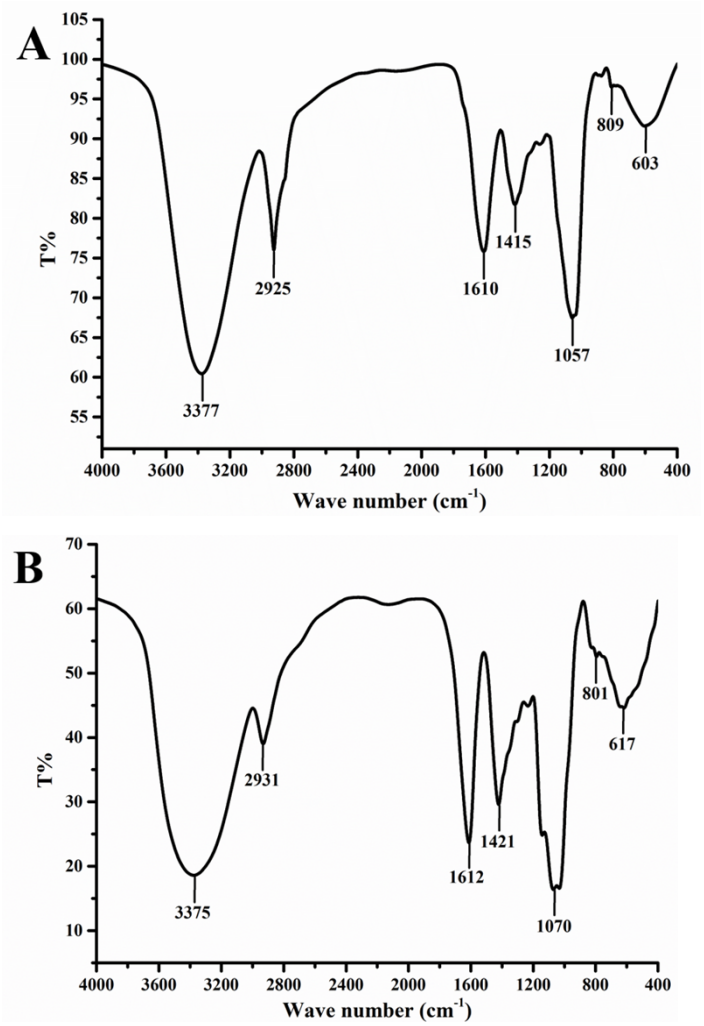


Fig. S5 FT-IR spectra of COP50-1 (A) and COP50-4 (B).

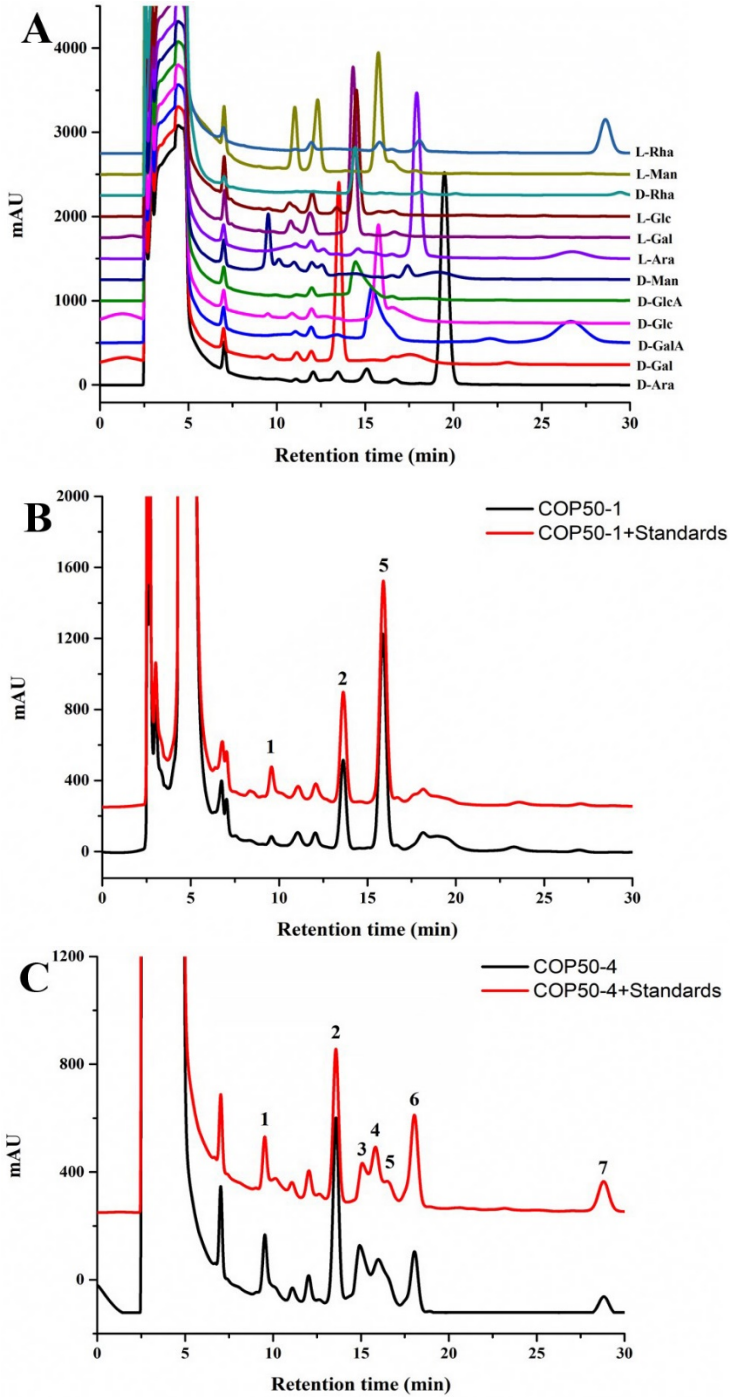
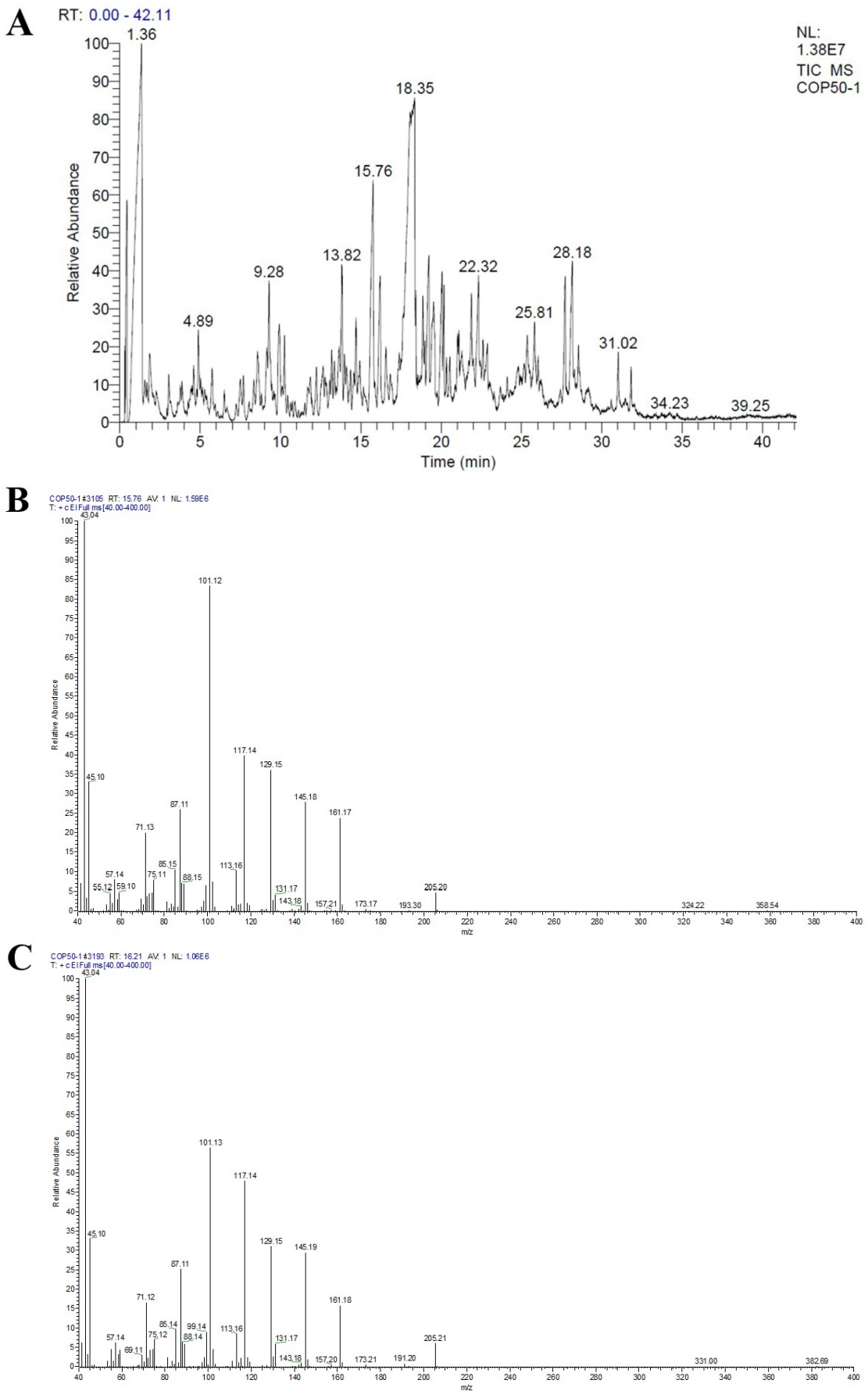
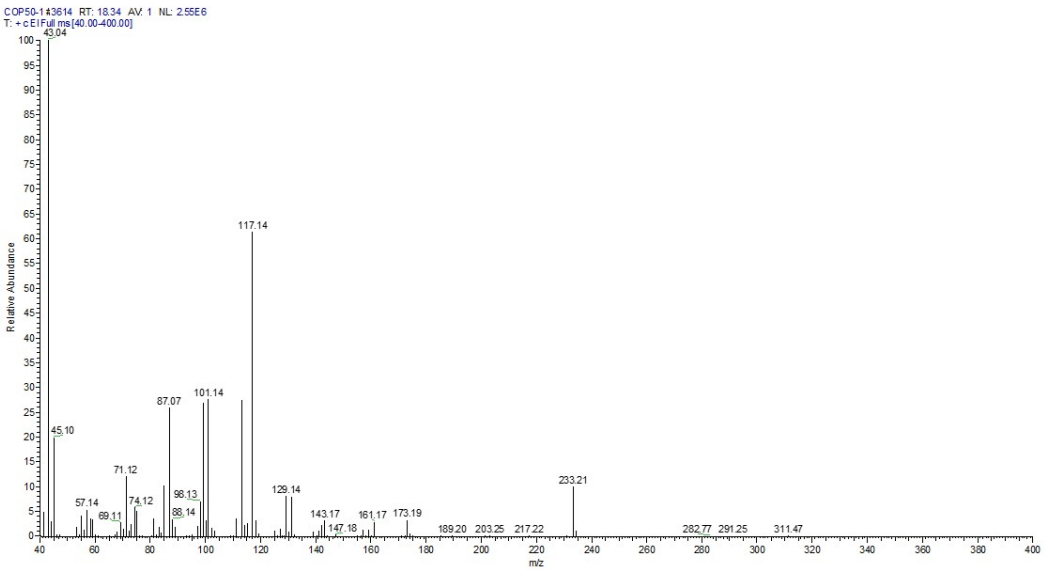


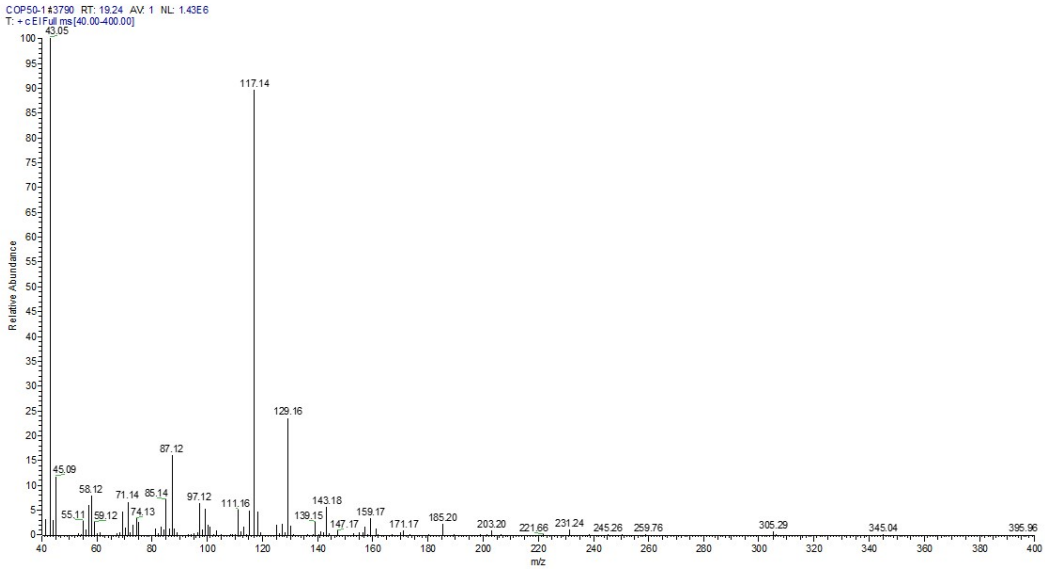
Fig. S6 HPLC chromatograms of (A) derivatives of standard monosaccharides, (B) COP50-1 derivatives and COP50-1 derivatives mixed with D-mannose, D-galactose, D-glucose and (C) COP50-4 derivatives and COP50-4 derivatives mixed with D-mannose, D-galactose, D-glucuronic acid, D-galactocuronic acid, D-glucose, L-arabinose, L-rhamnose.


A
RT: 0.00 - 42.11
NL:
1.38E7
TIC MS
COP50-1
Relative Abundance
Time (min)
1.36
4.89
9.28
13.82
15.76
18.35
22.32
25.81
28.18
31.02
34.23
39.25
B
COP50-1#3105 RT: 15.76 AV: 1 NL: 1.59E6
T: + c EI Full ms [40.00-400.00]
Relative Abundance
m/z
43.04
45.10
55.12
57.14
59.10
71.13
75.11
85.15
87.11
88.15
101.12
113.16
117.14
129.15
131.17
143.18
145.18
157.21
161.17
173.17
193.30
205.20
324.22
358.54
C
COP50-1#3193 RT: 16.21 AV: 1 NL: 1.06E6
T: + c EI Full ms [40.00-400.00]
Relative Abundance
m/z
43.04
45.10
57.14
69.11
71.12
75.12
85.14
87.11
88.14
99.14
101.13
113.16
117.14
129.15
131.17
143.18
145.19
157.20
161.18
173.21
191.20
205.21
331.00
382.69

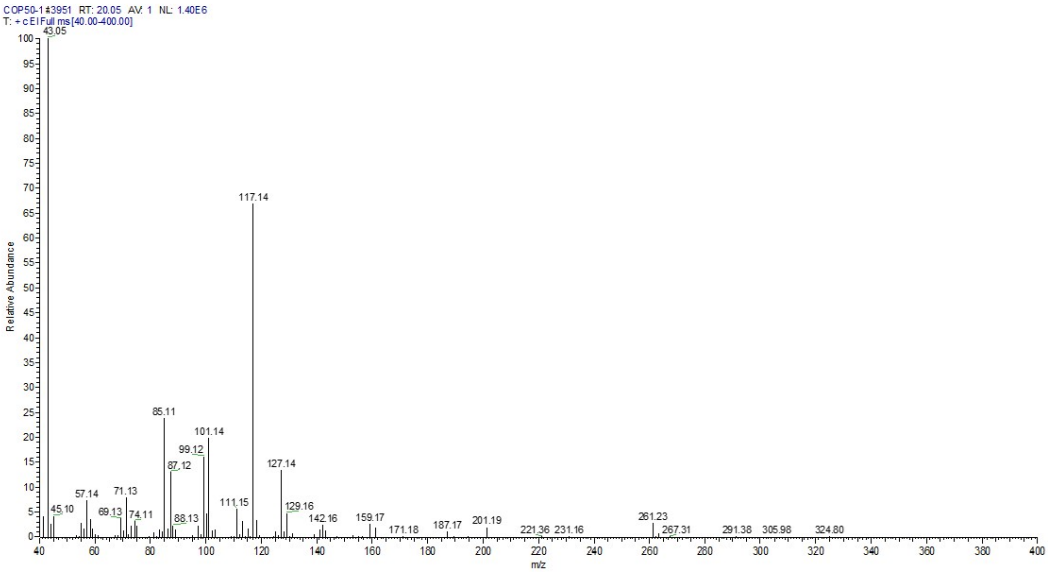
D


COP50-1 #3614 RT: 18.34 AV: 1 NL: 2.55E6
T: + c EI Full ms [40.00-400.00]
Relative Abundance
m/z
43.04
117.14
87.07
101.14
45.10
71.12
129.14
233.21
57.14
74.12
98.13
69.11
88.14
143.17
147.18
161.17
173.19
189.20
203.25
217.22
282.77
291.25
311.47

E


COP50-1 #3790 RT: 19.24 AV: 1 NL: 1.43E6
T: + c EI Full ms [40.00-400.00]
Relative Abundance
m/z
43.05
117.14
129.16
87.12
45.09
58.12
71.14
85.14
97.12
111.16
143.18
55.11
59.12
74.13
139.15
147.17
159.17
171.17
185.20
203.20
221.66
231.24
245.26
259.76
305.29
345.04
395.96

F


COP50-1 #3951 RT: 20.05 AV: 1 NL: 1.40E6
T: + c EI Full ms [40.00-400.00]
Relative Abundance
m/z
43.05
117.14
85.11
101.14
99.12
87.12
127.14
57.14
71.13
129.16
45.10
69.13
74.11
111.15
88.13
142.16
159.17
171.18
187.17
201.19
221.36
231.16
261.23
267.31
291.38
305.98
324.80

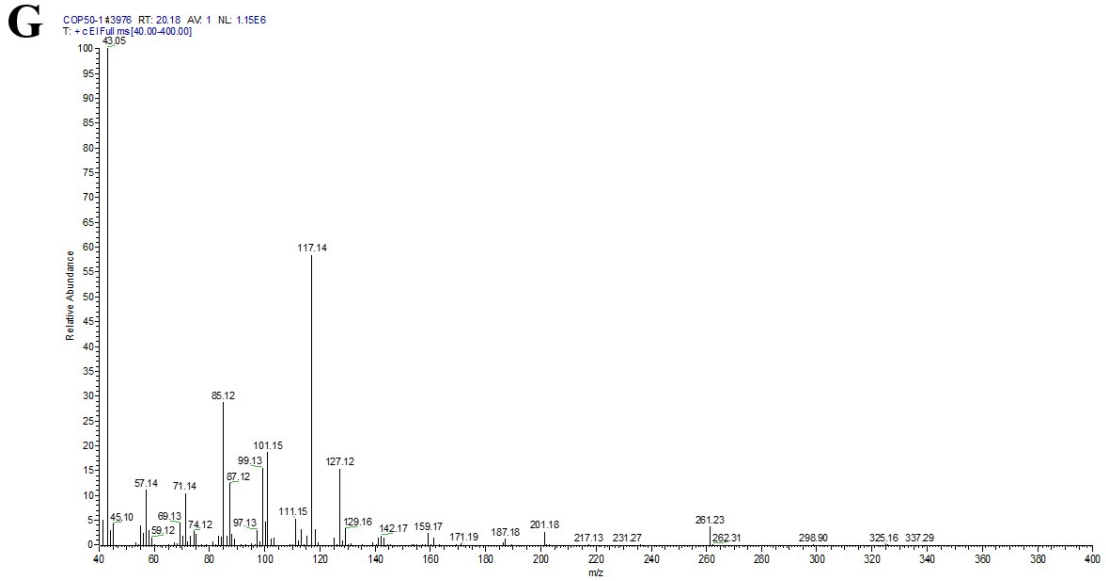


Fig. S7 A: The total ion chromatogram of COP50-1 derivates.

B: The mass spectrum of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol.

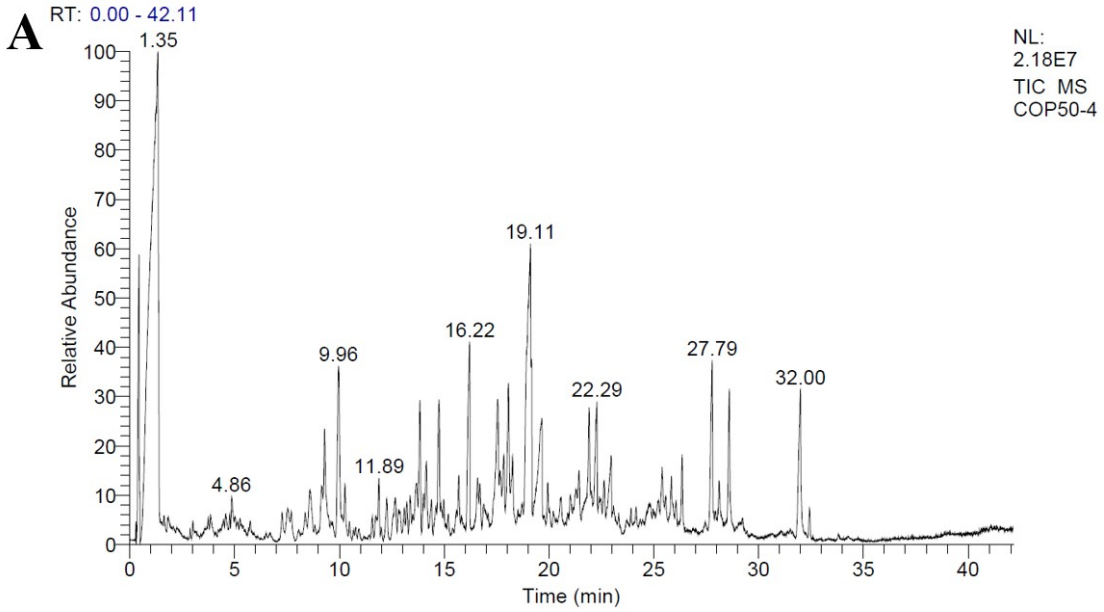
C: The mass spectrum of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-galactitol.

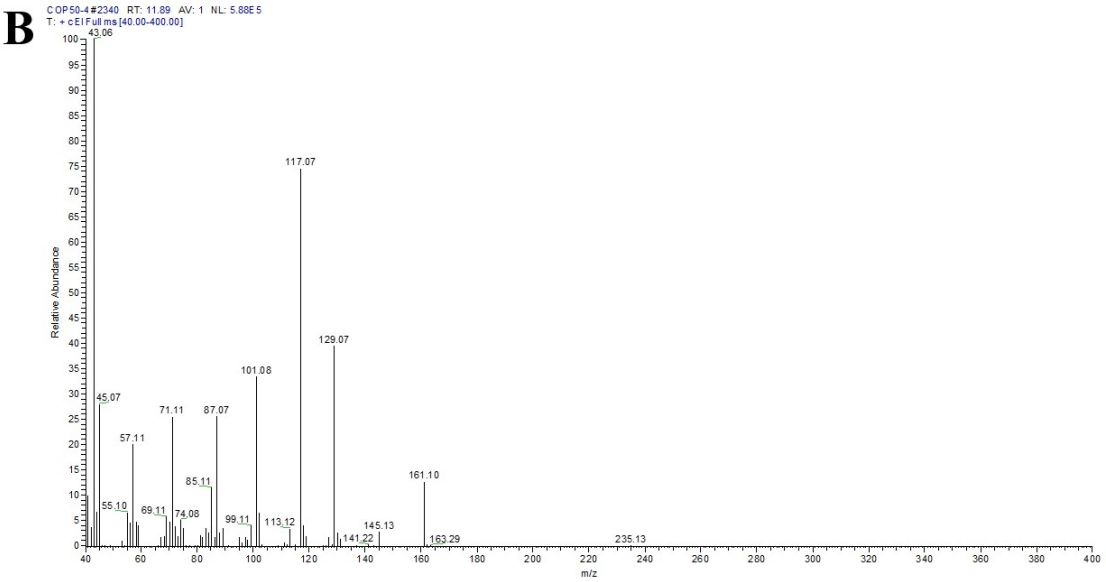
D: The mass spectrum of 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methyl-D-glucitol.

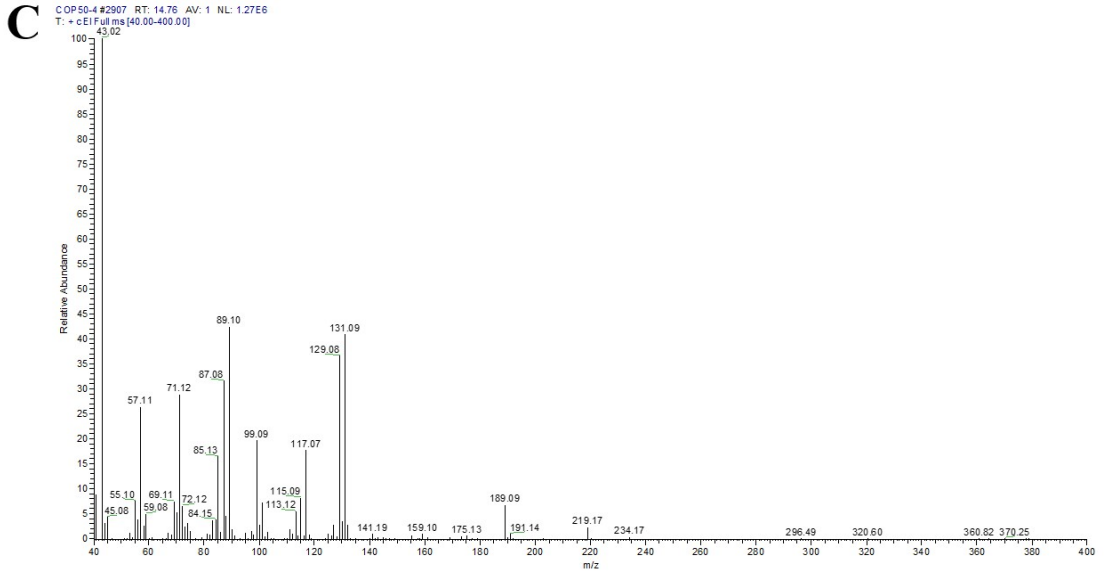
E: The mass spectrum of 1,3,4,5-tetra-*O*-acetyl-2,6-di-*O*-methyl-D-glucitol.

F: The mass spectrum of 1,4,5,6-tetra-*O*-acetyl-2,3-di-*O*-methyl-D-glucitol.

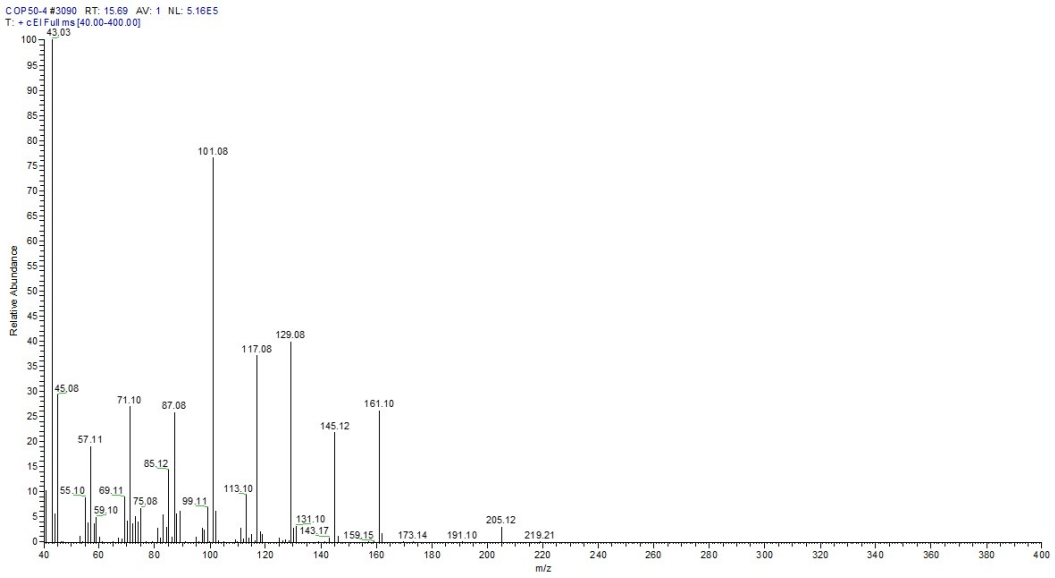
G: The mass spectrum of 1,4,5,6-tetra-*O*-acetyl-2,3-di-*O*-methyl-D-mannitol.


A
RT: 0.00 - 42.11
NL:
2.18E7
TIC MS
COP50-4
Relative Abundance
Time (min)
1.35
4.86
9.96
11.89
16.22
19.11
22.29
27.79
32.00


B
COP50-4 #2340 RT: 11.89 AV: 1 NL: 5.88E5
T: + c EI Full ms [40.00-400.00]
Relative Abundance
m/z
43.06
117.07
129.07
101.08
45.07
71.11
87.07
57.11
85.11
161.10
55.10
69.11
74.08
99.11
113.12
145.13
141.22
163.29
235.13


C
COP50-4 #2907 RT: 14.76 AV: 1 NL: 1.27E6
T: + c EI Full ms [40.00-400.00]
Relative Abundance
m/z
43.02
89.10
131.09
129.08
87.08
71.12
57.11
99.09
117.07
85.13
55.10
69.11
72.12
115.09
113.12
45.08
59.08
84.15
189.09
219.17
141.19
159.10
175.13
191.14
234.17
296.49
320.60
360.82
370.25

D


COP50-4 #3090 RT: 15.69 AV: 1 NL: 5.16E5
T: + c EI Full ms [40.00-400.00]
Relative Abundance
m/z
43.03
101.08
129.08
117.08
45.08
71.10
87.08
161.10
145.12
57.11
85.12
55.10
69.11
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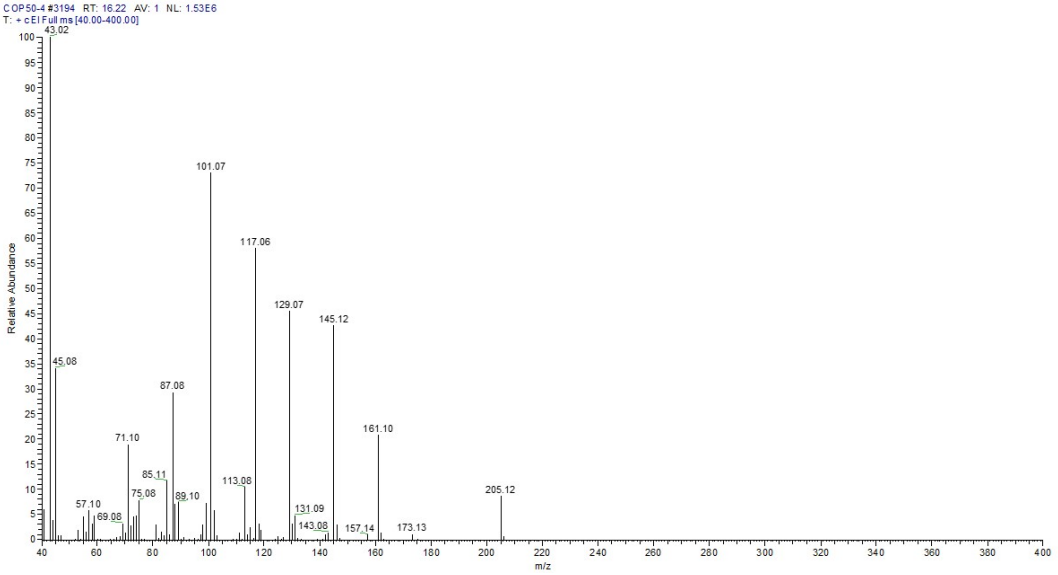
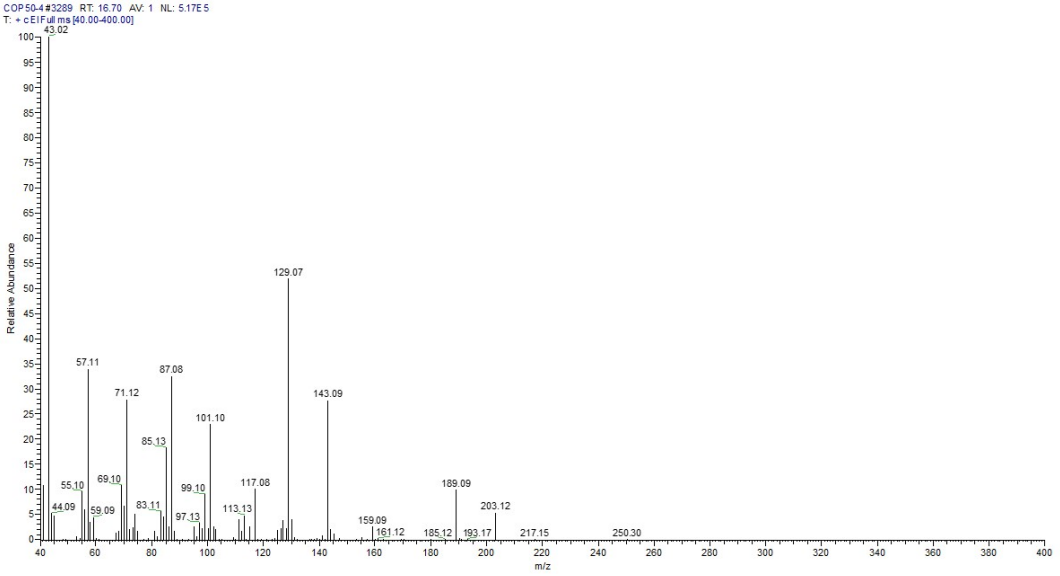
143.17
159.15
173.14
191.10
205.12
219.21

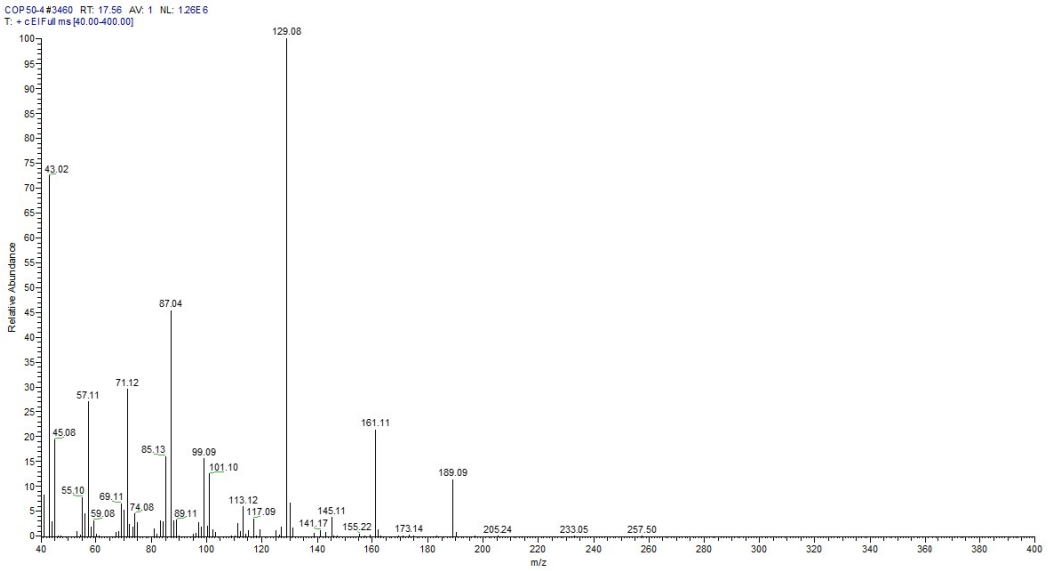
E


COP50-4 #3194 RT: 16.22 AV: 1 NL: 1.53E6
T: + c EI Full ms [40.00-400.00]
Relative Abundance
m/z
43.02
101.07
117.06
129.07
145.12
45.08
87.08
161.10
71.10
85.11
113.08
205.12
57.10
75.08
89.10
131.09
69.08
143.08
157.14
173.13

F


COP50-4 #3289 RT: 16.70 AV: 1 NL: 5.17E5
T: + c EI Full ms [40.00-400.00]
Relative Abundance
m/z
43.02
129.07
57.11
87.08
143.09
71.12
101.10
85.13
55.10
69.10
99.10
117.08
189.09
44.09
59.09
83.11
97.13
113.13
203.12
159.09
161.12
185.12
193.17
217.15
250.30

G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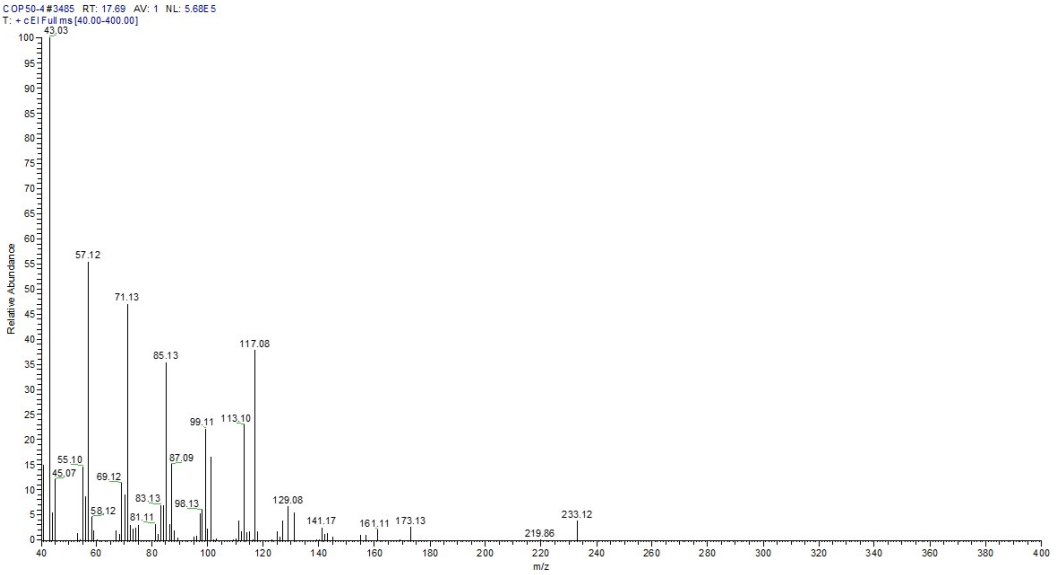
Relative Abundance
m/z
129.08
43.02
87.04
71.12
57.11
161.11
45.08
85.13
99.09
101.10
189.09
55.10
69.11
74.08
113.12
59.08
89.11
117.09
145.11
141.17
155.22
173.14
205.24
233.05
257.50

H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Relative Abundance
m/z
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57.12
71.13
117.08
85.13
99.11
113.10
55.10
87.09
45.07
69.12
83.13
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141.17
161.11
173.13
219.86
233.12

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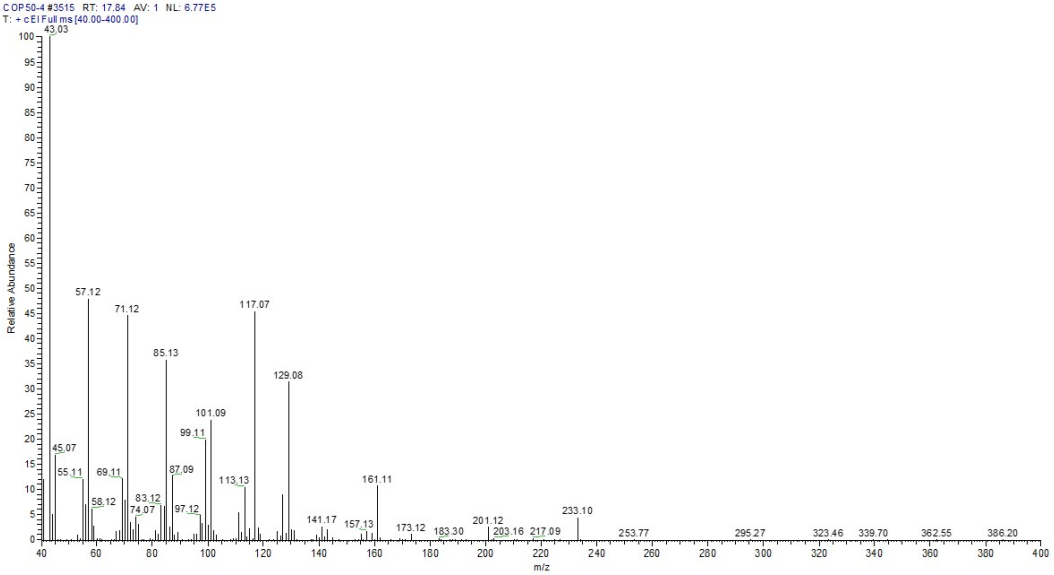
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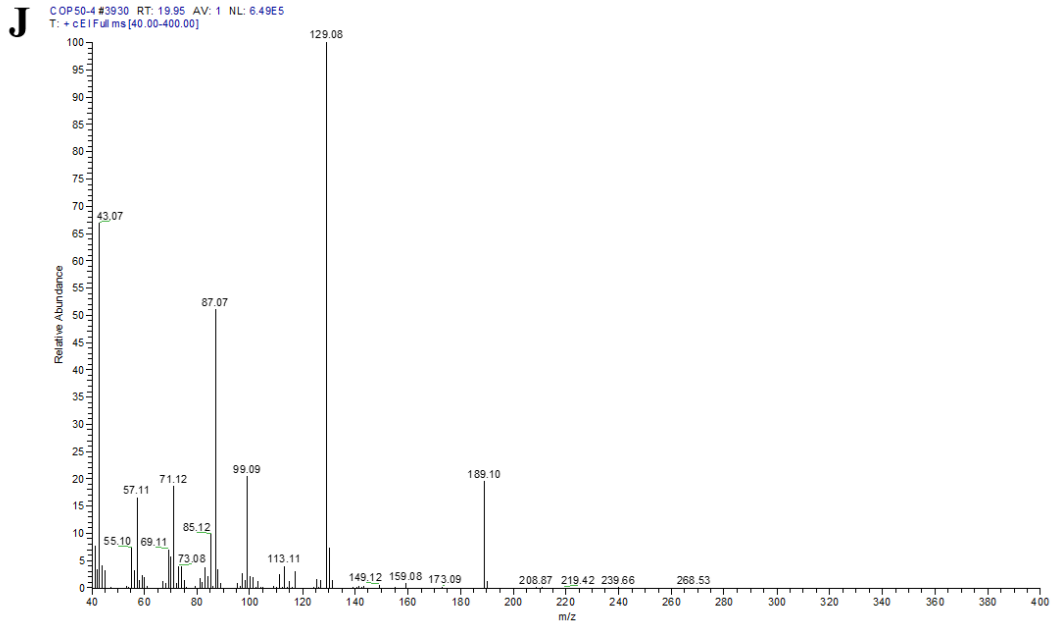
T: + c EI Full ms [40.00-400.00]
Relative Abundance
m/z
43.03
57.12
71.12
117.07
85.13
129.08
101.09
99.11
45.07
55.11
69.11
87.09
113.13
161.11
58.12
83.12
97.12
74.07
141.17
157.13
173.12
183.30
201.12
203.16
217.09
233.10
253.77
295.27
323.46
339.70
362.55
386.20

J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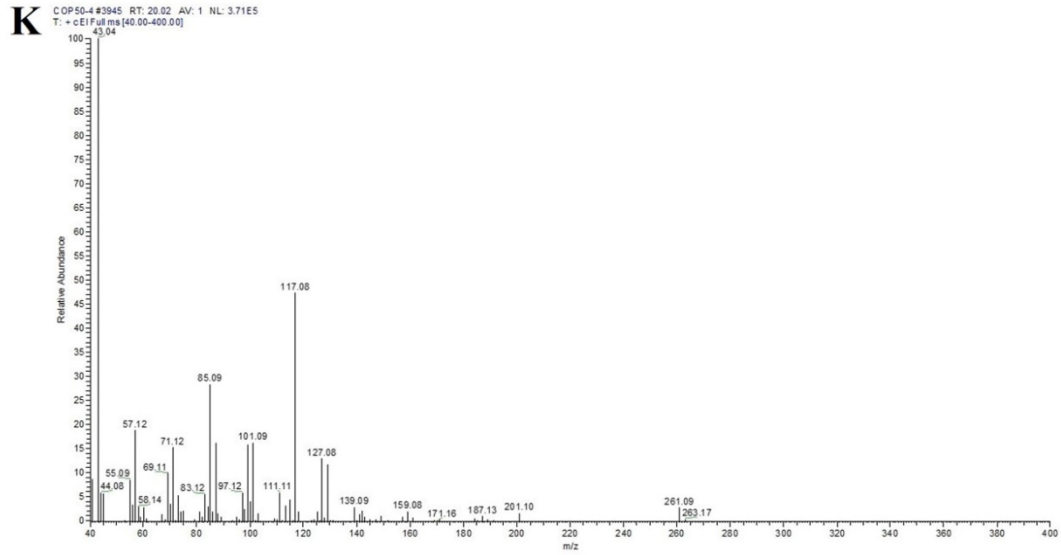
T: + c EI Full ms [40.00-400.00]
Relative Abundance
m/z
129.08
43.07
87.07
99.09
189.10
57.11
71.12
85.12
55.10
69.11
73.08
113.11
149.12
159.08
173.09
208.87
219.42
239.66
268.53

K


COP50-4 #3945 RT: 20.02 AV: 1 NL: 3.71E5
T: + c EI Full ms [40.00-400.00]
Relative Abundance
m/z
43.04
117.08
85.09
57.12
71.12
101.09
127.08
55.09
69.11
44.08
83.12
97.12
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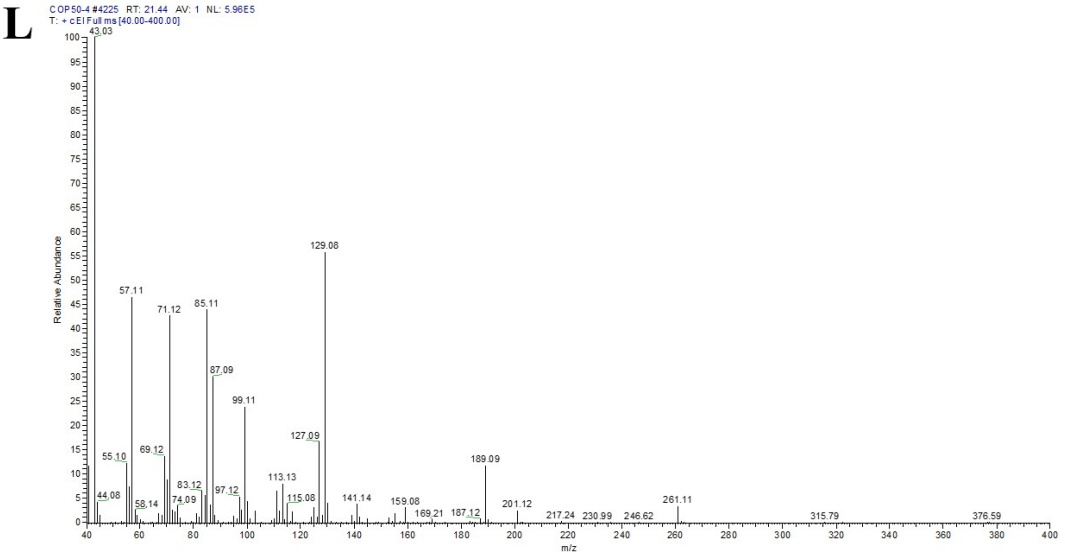
171.16
187.13
201.10
261.09
263.17

L


COP50-4 #4225 RT: 21.44 AV: 1 NL: 5.96E5
T: + c EI Full ms [40.00-400.00]
Relative Abundance
m/z
43.03
129.08
57.11
71.12
85.11
87.09
99.11
127.09
189.09
55.10
69.12
113.13
83.12
97.12
115.08
44.08
58.14
74.09
141.14
159.08
169.21
187.12
201.12
217.24
230.99
246.62
261.11
315.79
376.59

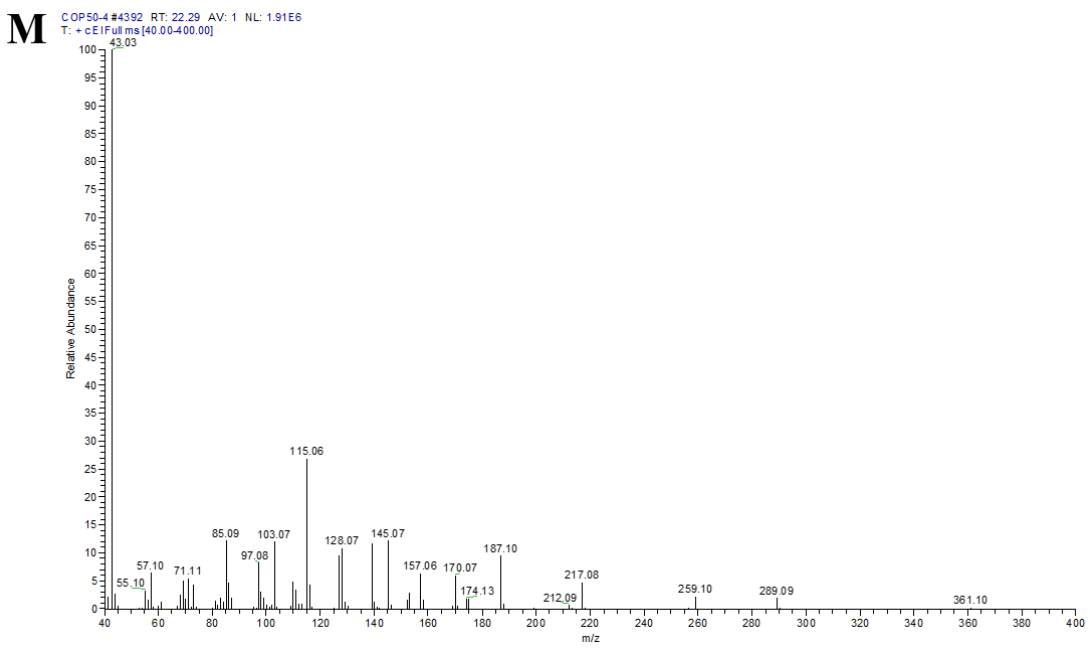


Fig. S8 A: The total ion chromatogram of COP50-4 derivates.

B: The mass spectrum of 1,4-di-*O*-acetyl-2,3,5-tri-*O*-methyl-L-arabinitol.

C: The mass spectrum of 1,2,5-tri-*O*-acetyl-6-deoxy-3,4-di-*O*-methyl-L-mannitol.

D: The mass spectrum of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-mannitol.

E: The mass spectrum of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-galactitol.

F: The mass spectrum of 1,2,4,5-tetra-*O*-acetyl-6-deoxy-3-*O*-methyl-L-mannitol.

G: The mass spectrum of 1,2,5-tri-*O*-acetyl-3,4,6-tri-*O*-methyl-D-mannitol.

H: The mass spectrum of 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methyl-D-glucitol.

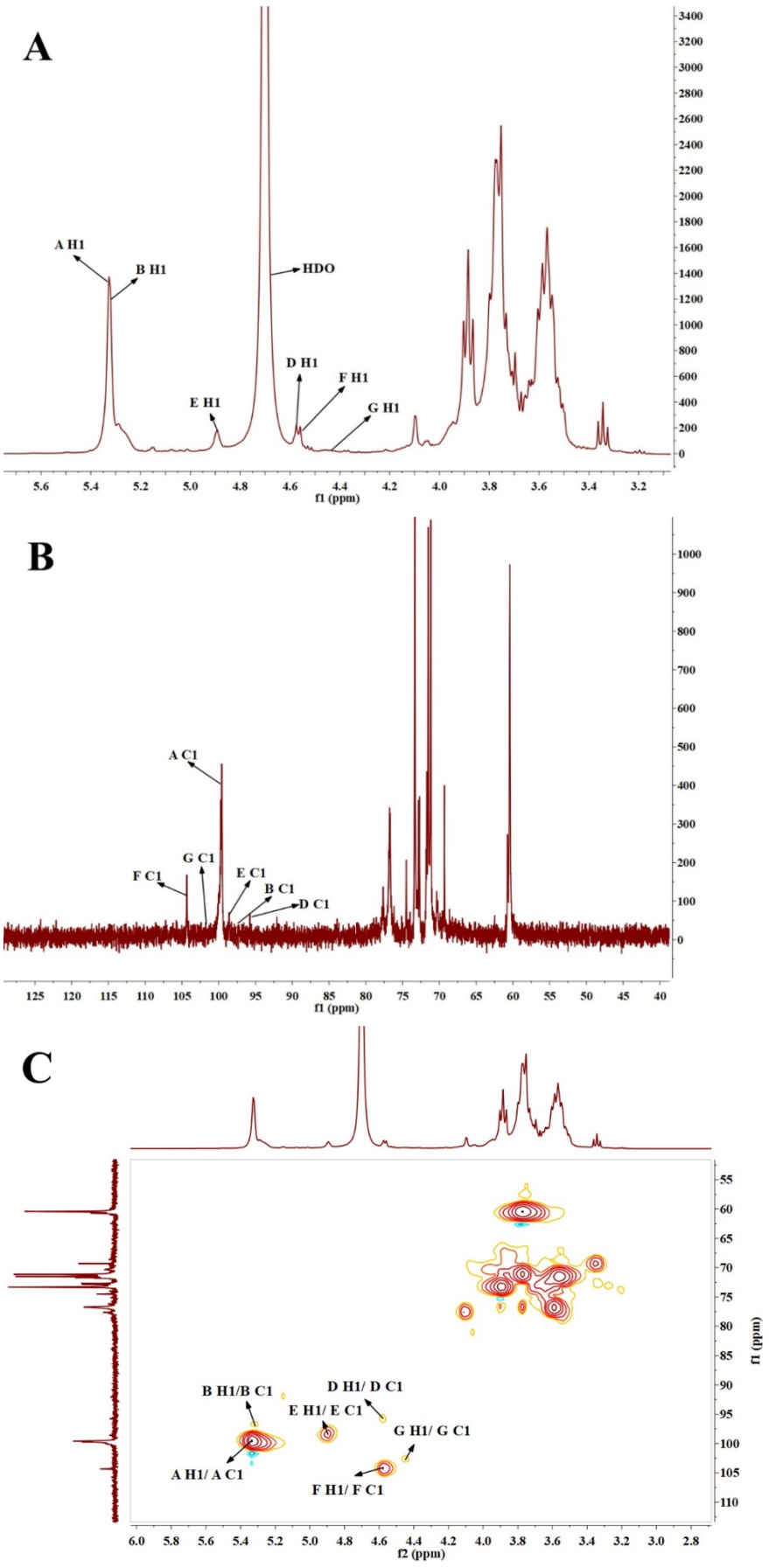
I: The mass spectrum of 1,3,5-tri-*O*-acetyl-2,4,6-tri-*O*-methyl-D-galactitol.

J: The mass spectrum of 1,2,4,5-tetra-*O*-acetyl-3-*O*-methyl-L-arabinitol.

K: The mass spectrum of 1,4,5,6-tetra-*O*-acetyl-2,3-di-*O*-methyl-D-galactitol.

L: The mass spectrum of 1,2,3,5,6-penta-*O*-acetyl-4-*O*-methyl-D-mannitol.

M: The mass spectrum of 1,2,3,4,5-penta-*O*-acetyl-L-arabinitol.


A
A H1
B H1
HDO
D H1
F H1
E H1
G H1
f1 (ppm)
B
A C1
G C1
F C1
E C1
B C1
D C1
f1 (ppm)
C
B H1/B C1
D H1/ D C1
E H1/ E C1
G H1/ G C1
A H1/ A C1
F H1/ F C1
f2 (ppm)
f1 (ppm)

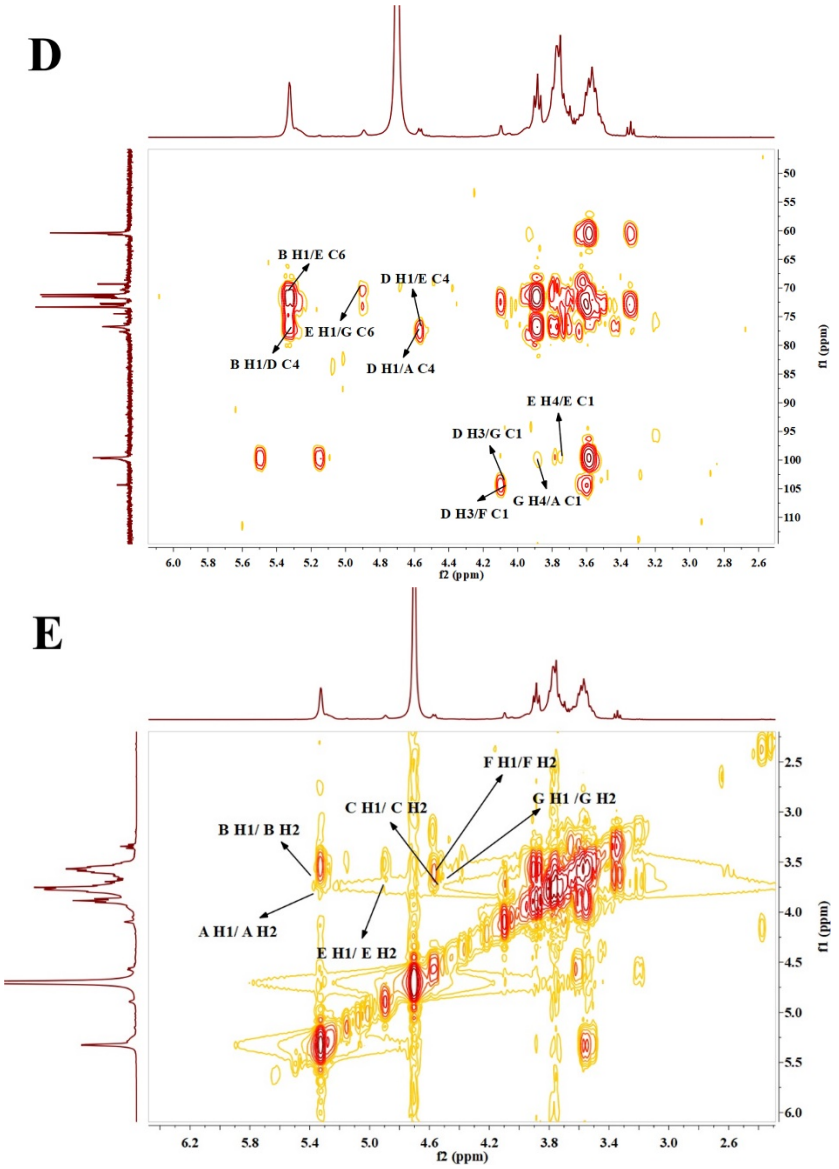


Fig. S9 (A) ^{1}H-NMR, (B) ^{13}C-NMR, (C) HSQC, (D) HMBC, and (E) ^{1}H–^{1}H COSY spectra of COP50-1.

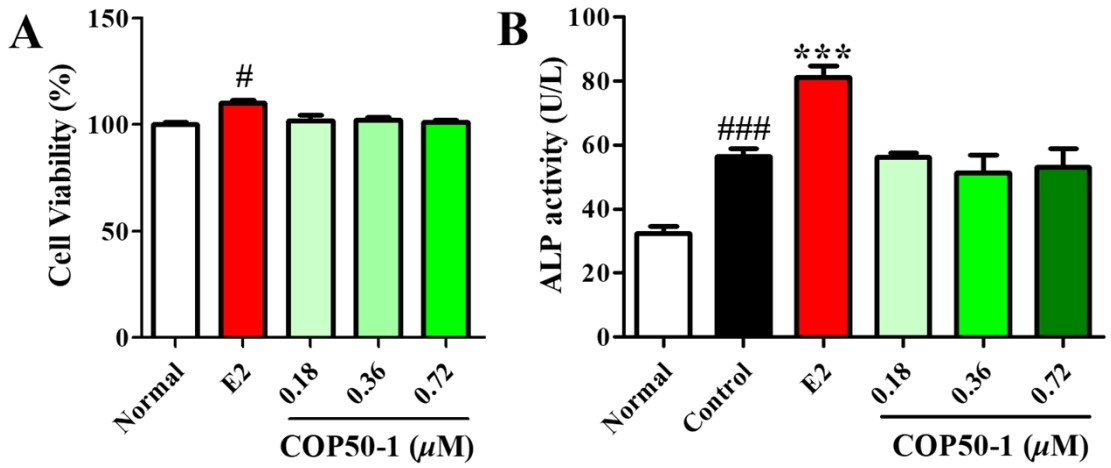


Fig. S10 Effects of COP50-1 on the proliferation (A) and differentiation (B) of MC3T3-E1 cells. Values are mean ± SEM. $^{\#}P < 0.05$, $^{\#\#\#}P < 0.001$ *vs.* Normal. $^{***}P < 0.001$ *vs.* Control.