Electronic Supplementary Material (ESI) for Green Chemistry.

Supplementary Information

New degradation compounds from lignocellulosic biomass pretreatment: Routes for formation of potent oligophenolic enzyme inhibitors

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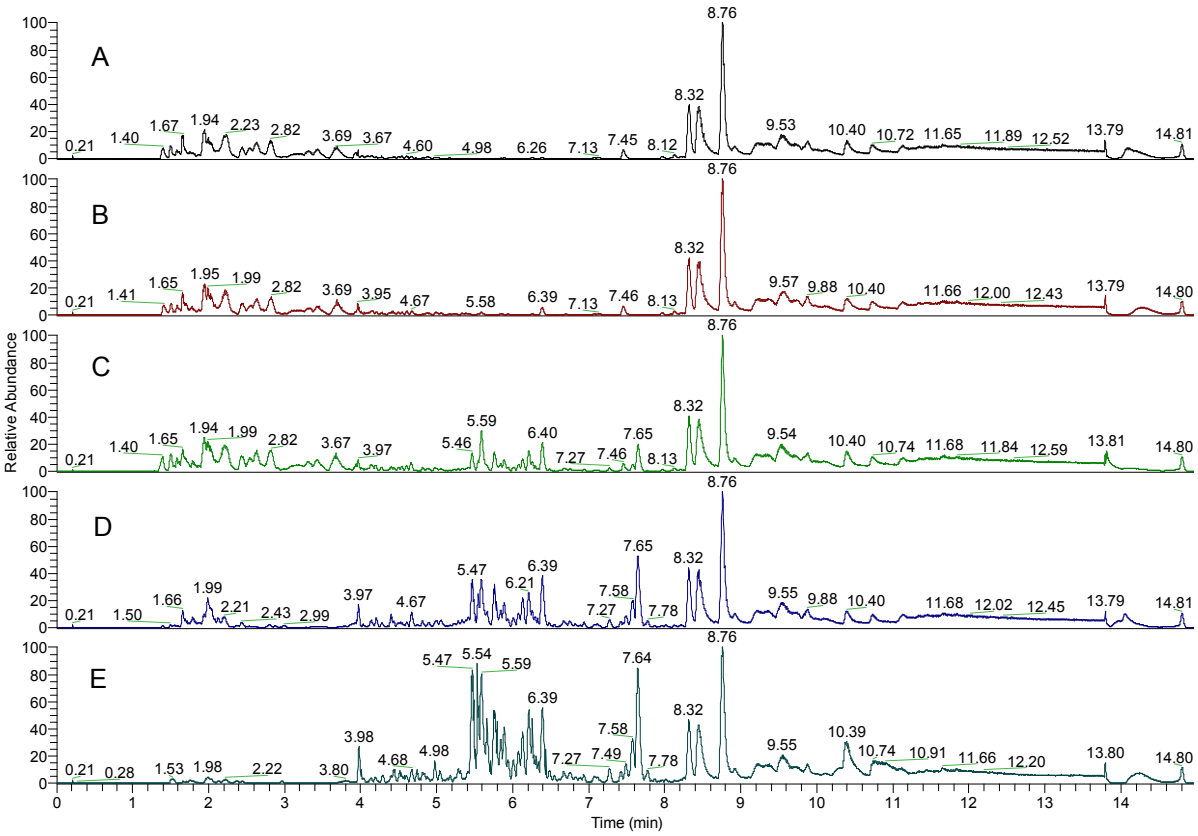


S1 Figure 1. Base peak chromatograms (negative ionisation) of fractions from solvent extractions. A: Water fraction from 2-butanol extraction. B: Water fraction from 2-butanone extraction. C: Non extracted LfHP. D: 2-butanol fraction. E: 2-butanone fraction. The fractions exhibit increasing inhibition from top to bottom

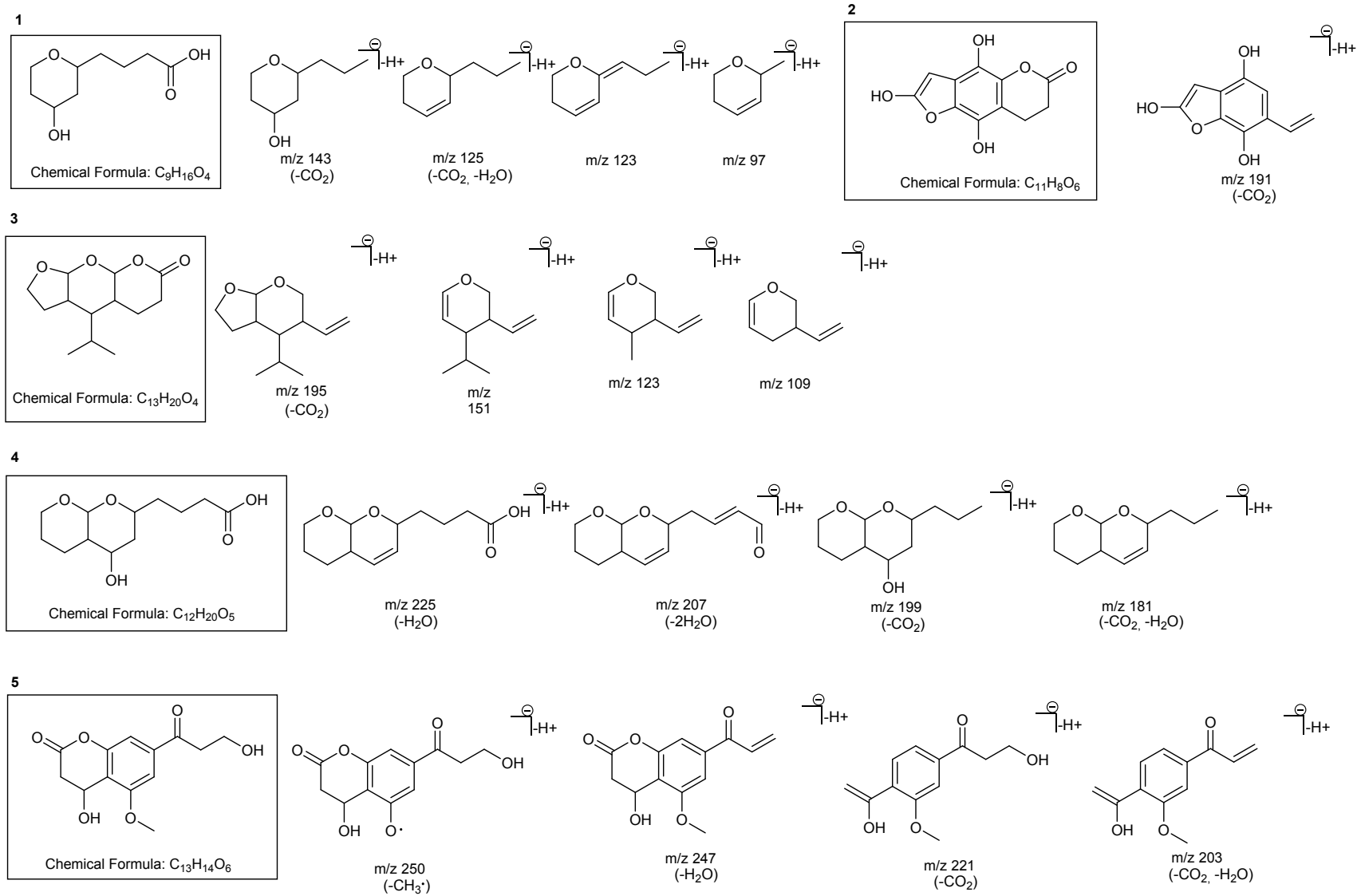
S1 Table 1. LC-MS/MS negative ionisation, 2-butanone fraction. Fragment structures are proposed in Table 3.

Entry	Retention time (min)	Accurate mass	C	H	O	DBE	Precursor ion (intensity)	Fragments (intensity)												
1	6.25-6.27	188,10433	9	16	4	2	187,09705 (14)	143,10754 (2)	125,09683 (100)	123,08135 (8)	97,06552 (18)									
2	6.31-6.33	236,03176	11	8	6	8	235,02448 (38)	191,03442 (100)												
3	6.37-6.42	240,13576	13	20	4	4	239,12848 (38)	195,13854 (100)	151,1123 (36)	123,08097 (18)	109,06533 (14)									
4	6.46-6.48	244,13060	12	20	5	3	243,12332 (30)	225,11264 (100)	207,10204 (62)	199,13336 (18)	181,12275 (30)	163,11216 (10)	99,00808 (20)							
5	5.53-5.63	266,07894	13	14	6	7	265,07166 (10)	250,04776 (3)	247,06071 (4)	221,08136 (12)	203,07075 (8)	193,04996 (100)	178,02649 (66)	175,03944 (14)	160,01592 (8)	149,06008 (40)	134,0366 (68)			
6	6.28-6.30	276,09953	15	16	5	8	275,09225 (24)	231,1023 (100)	216,07859 (30)	215,07114 (66)	203,07103 (10)	188,04753 (9)	107,04969 (10)							
7	6.04-6.09	294,10997	15	18	6	7	293,10269 (4)	275,09254 (8)	249,11306 (12)	231,10243 (24)	216,07891 (28)	163,07601 (10)	149,06028 (40)	134,03677 (18)	131,03439 (26)	130,02661 (88)	125,06032 (20)	123,04469 (34)	113,06021 (100)	99,0446 (20)
8	5.44-5.52	296,08956	14	16	7	7	295,08228 (2)	235,06073 (16)	205,05010 (4)	163,03936 (100)	145,02881 (38)	119,04955 (36)								
9	7.26-7.30	302,07866	16	14	6	10	301,07138 (66)	286,04786 (100)	268,03753 (8)	257,04529 (4)										
10	6.25-6.27	306,11006	16	18	6	8	305,10278 (26)	290,07926 (8)	261,11277 (100)	246,0893 (18)	245,08158 (20)	231,0658 (82)								
11	6.06-6.09	308,05290	14	12	8	9	307,04562 (100)	289,03492 (18)	263,05571 (36)	245,04506 (18)	219,06572 (48)	201,05522 (4)								
12	5.53-5.63	326,09981	15	18	8	7	325,09253 (1)	265,07184 (20)	235,06097 (7)	193,05008 (100)	175,03971 (32)	160,01625 (6)	149,06035 (14)	134,03681 (16)						
13	5.78-5.80	328,09429	18	16	6	11	327,08701 (10)	283,09729 (20)	239,10756 (8)	163,03944 (100)	119,04959 (42)									
14	5.92-5.97	328,09429	18	16	6	11	327,08701 (100)	309,07652 (20)	283,09722 (22)	265,08674 (74)	237,09171 (20)	211,07598 (16)	163,03945 (29)	143,03945 (29)	119,04957 (15)	93,03396 (21)				
15	7.63-7.70	330,07509	17	14	7	11	329,06781 (40)	314,04321 (100)	299,02028 (40)											
16	6.56-6.60	342,10994	19	18	6	11	341,10266 (40)	326,07909 (82)	323,09197 (8)	311,05567 (12)	309,07647 (48)	297,11269 (36)	294,05306 (62)	282,08942 (100)	267,06603 (72)					
17	6.58-6.64	354,11003	20	18	6	12	353,10275 (20)	338,07918 (100)	323,05553 (60)	310,08417 (10)	294,08935 (36)	279,06586 (38)								
18	5.98-6.02	358,10515	19	18	7	11	357,09785 (100)	339,08689 (10)	313,10771 (44)	295,09717 (25)	280,07378 (17)	254,09451 (12)	193,05011 (10)	163,03949 (12)	119,04959 (16)					
19	6.60-6.65	372,12044	20	20	7	11	371,11316 (26)	356,09000 (65)	341,06621 (26)	339,08698 (30)	327,12339 (29)	324,06356 (25)	312,10001 (100)	297,07654 (64)	294,08957 (9)	279,06603 (6)				
20	5.92-5.97	372,12053	20	20	7	11	371,11325 (21)	356,08998 (14)	353,10282 (42)	341,10258 (100)	327,12328 (88)	326,07928 (31)	312,09995 (87)	309,11293 (36)	297,11276 (12)	294,08948 (66)	282,08945 (24)	279,06596 (40)	173,06023 (27)	123,04453 (12)
21	7.41-7.43	384,12044	21	20	7	12	383,11316 (62)	368,08932 (100)	353,06612 (9)	229,05020 (9)										
22	6.60-6.65	384,12062	21	20	7	12	383,11334 (14)	368,08959 (54)	353,06676 (100)	352,09528 (60)	338,07910 (32)	337,07167 (92)	324,09976 (7)	309,07644 (16)						
23	5.78-5.80	388,11531	20	20	8	11	387,10803 (100)	369,09787 (100)	354,07428 (30)	343,11832 (35)	325,10786 (70)	310,08443 (65)	295,06082 (12)	282,08945 (43)	193,05017 (65)	173,06131 (21)	159,04464 (15)	149,06022 (24)	134,03673 (23)	123,0445 (14)
24	5.84-5.91	388,11540	20	20	8	11	387,10812 (20)	343,11857 (30)	299,12878 (4)	284,10534 (9)	193,05013 (100)	178,02676 (5)	149,06028 (12)	134,03679 (22)						
25	5.92-5.97	388,11546	20	20	8	11	387,10818 (100)	369,09748 (18)	354,07416 (5)	343,11805 (6)	325,10755 (30)	310,08415 (25)	282,08933 (15)	271,09741 (5)	193,05001 (20)	173,06023 (17)	159,04457 (8)	134,03663 (5)	123,04451 (7)	
26	5.63-5.73	402,13097	21	22	8	11	401,12369 (26)	386,10059 (17)	383,11344 (45)	371,11343 (100)	357,13426 (95)	342,11047 (87)	339,12345 (36)	327,12299 (10)	324,09995 (66)	312,10007 (25)	309,07664 (43)	217,05026 (10)	203,03457 (3)	173,06027 (24)
27	7.77-7.80	480,13978	26	24	9	15	479,1325 (100)	464,11117 (10)	449,08776 (4)	355,08239 (41)	340,05855 (6)									
28	7.29-7.36	508,13628	27	24	10	16	507,12900 (100)	492,10619 (36)	477,08256 (5)	341,06650 (24)										
29	6.40-6.45	528,12630	26	24	12	15	527,11902 (69)	509,10795 (6)	329,06609 (100)	185,04494 (24)	167,03436 (2)									
30	6.94-6.99	548,16805	30	28	10	17	547,16077 (3)	503,17078 (6)	401,12355 (44)	383,11315 (100)	371,11332 (16)	368,0899 (13)	357,13427 (12)	339,12312 (60)	324,09977 (56)	309,07643 (8)	163,03948 (15)	145,02882 (79)	119,04960 (6)	117,03393 (9)

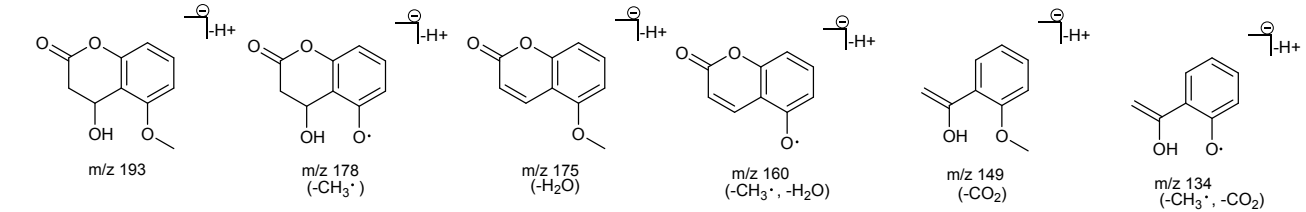
S1 Table 2. LC-MS/MS negative ionisation, water fraction from 2-butanone extraction. Shaded fragments are in common with LC-MS/MS fragments from xylooligosaccharide standards(xylobiose to xylohexaose).

Retention Time (min)	Accurate mass	C	H	O	DBE	Compound	Fragments														
1.97-1.99	150,05203	5	10	5	1	xylose	131,03508	129,01977	101,02457	85,02951											
1.85-1.87	180,06282	6	12	6	1	glucose	161,04527	150,95383	134,98767	131,03461	122,95918	113,02392	101,02410	97,02912	89,02398	85,02911					
1.79-1.81	282,09505	10	18	9	2	xylobiose	203,05728	131,03503	112,98583	101,02453	85,02950										
1.91-1.96	460,14241	16	28	15	3	xylotriose	335,09823	263,07716	203,05581	149,04506	131,03442	113,02387	101,02386	85,02897							
3.94-3.96	502,15434	18	30	16	4	1 acetyl xylotriose	377,11137	335,10167	305,08741	263,07894	149,04559	131,03513	113,02467	101,02460	85,02948						
2.75-2.77	546,17928	20	34	17	4	xylotetraose	467,14017	395,11896	335,09780	263,07701	203,05563	149,04497	131,03436	113,02380	101,02379	85,02890					
3.94-3.96	634,19679	23	38	20	5	1 acetyl xylotetraose	527,16246	509,15079	467,14007	437,12956	395,11893	377,10861	335,09898	305,08789	263,07776	203,05563	149,04497	131,03436	113,02380	101,02379	85,02890
3.13-3.21	678,22157	25	42	21	5	xylopentaose	527,16135	467,14013	395,11896	263,07702	131,03437	101,02381									
3.94-3.96	766,23934	28	46	24	6	1 acetyl xylotepentaose	641,19406	599,18158	569,17220	509,15079	467,14007	437,12956	395,11893	377,10861	305,08789	263,07776					
3.31-3.35	810,26436	30	50	25	6	xylohexaose	731,22514	719,22522	659,20411	641,19357	623,18283	611,18273	599,18294	527,16164	509,15109	467,14041	395,11922	377,10893	263,07721		
4.00-4.03	894,28456	34	54	27	8	2 acetyl xylohexaose	731,22514	719,22522	659,20411	641,19357	623,18283	611,18273	599,18294	527,16164	509,15109	467,14041	395,11922	377,10893	263,07721		

S1 Table 3. Proposed MS/MS fragments (*continued*).



S1 Table 3 (*continued*). Proposed MS/MS fragments (*continued*).



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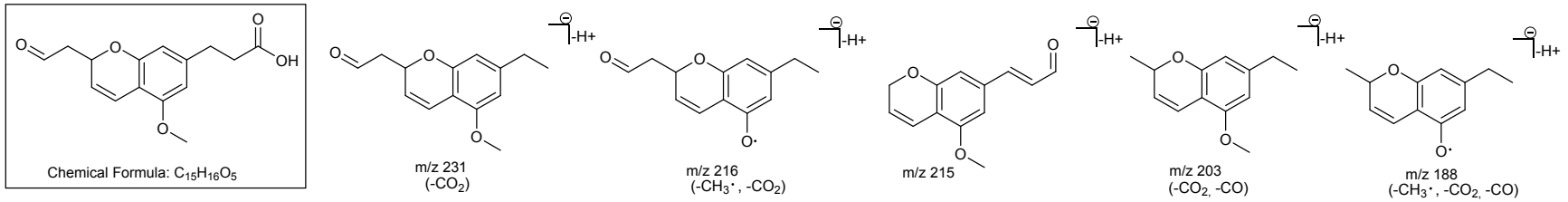

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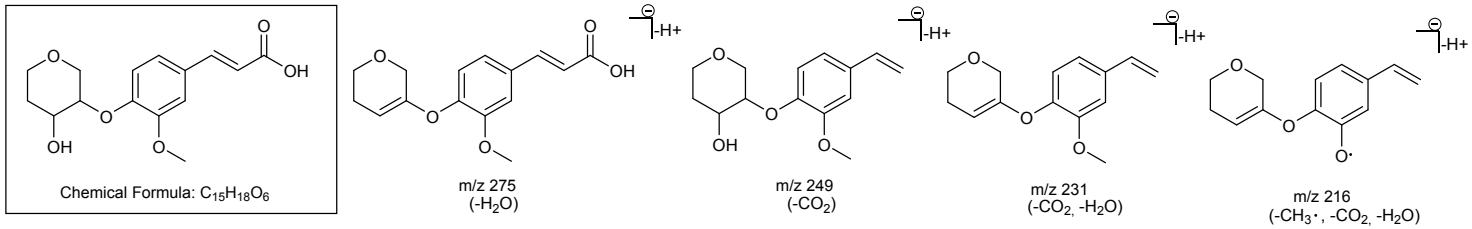

S1 Table 3 (*continued*). Proposed MS/MS fragments (*continued*).

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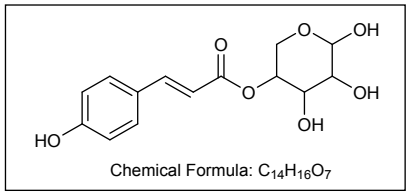

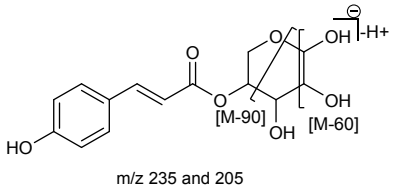

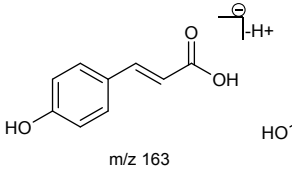

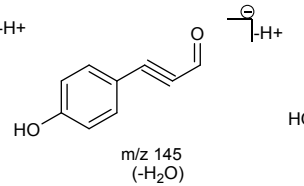

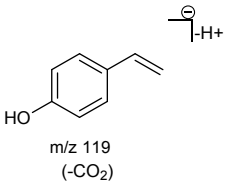

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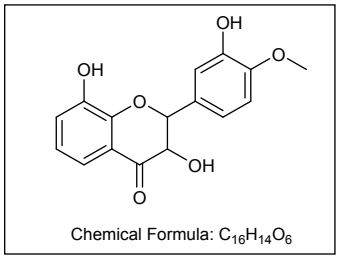

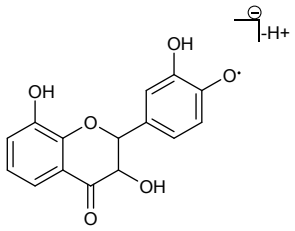

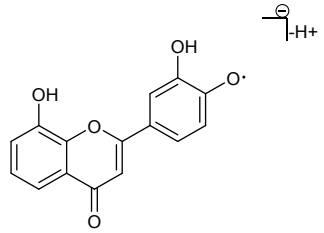

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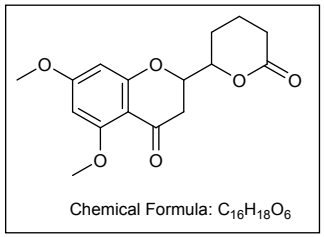

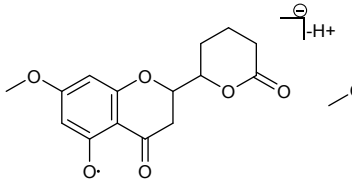

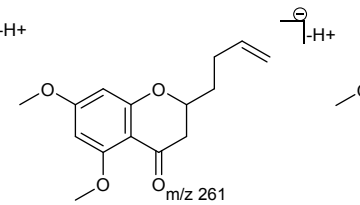

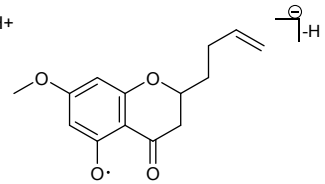

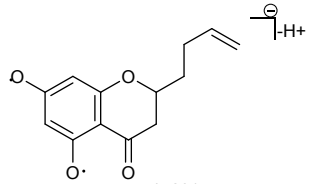

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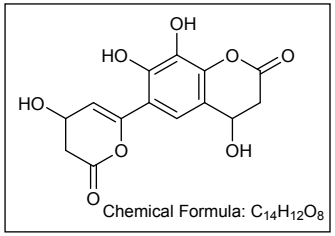

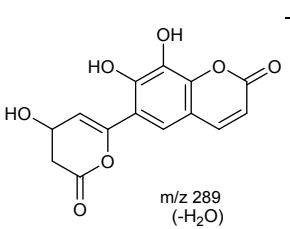

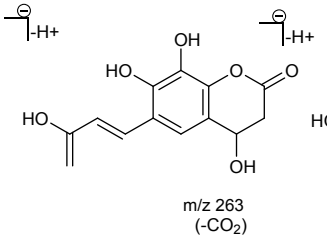

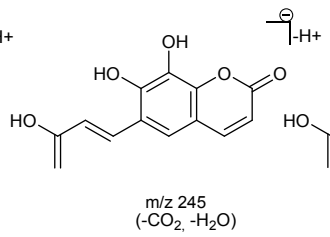

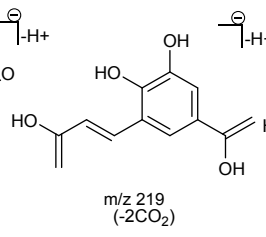

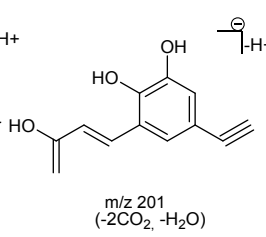

S1 Table 3 (*continued*). Proposed MS/MS fragments (*continued*).

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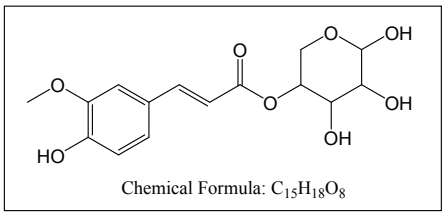

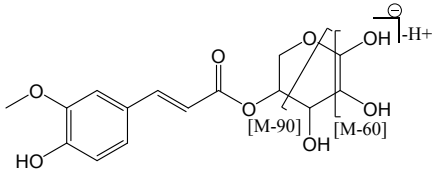

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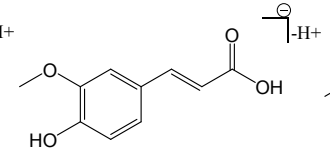

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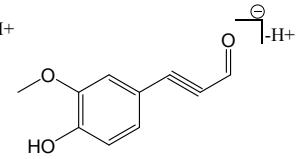

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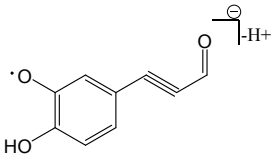

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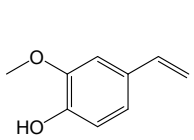
($-CH_3^{\bullet}$, $-H_2O$)



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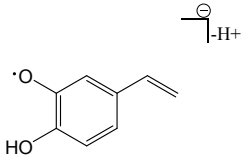
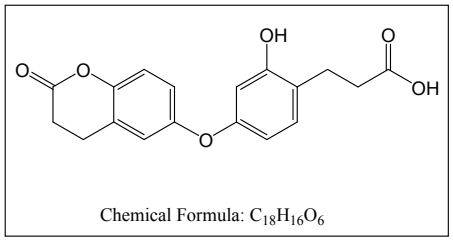
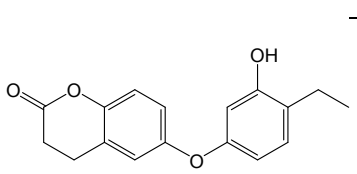
($-CO_2$)



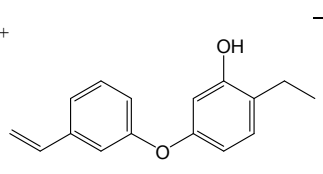
m/z 134
($-CH_3^{\bullet}$, $-CO_2$)

13

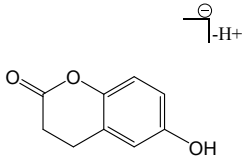




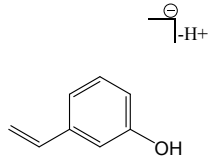
m/z 283
($-CO_2$)



m/z 239
($-2CO_2$)

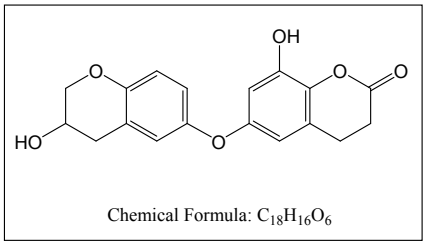


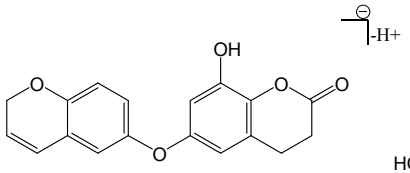
m/z 163



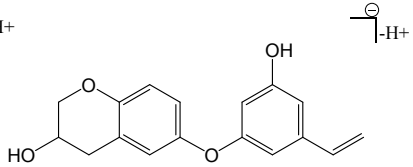
m/z 119

14

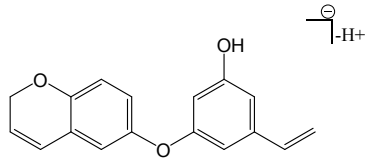




m/z 309
($-H_2O$)

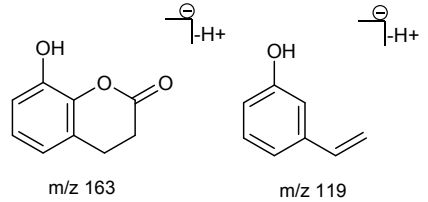


m/z 283
($-CO_2$)

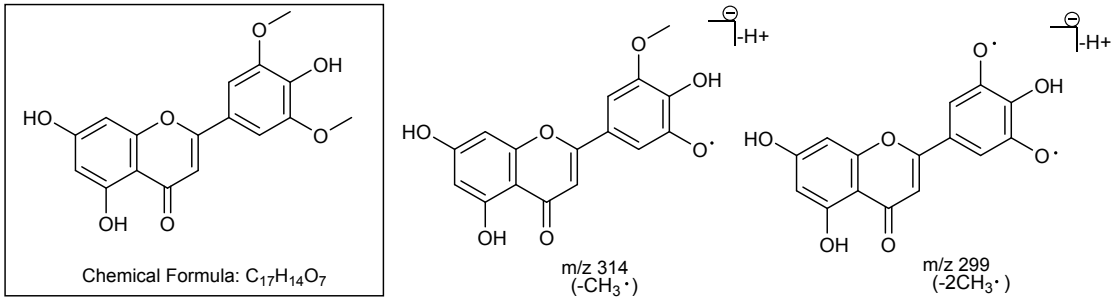


m/z 265
($-CO_2$, $-H_2O$)

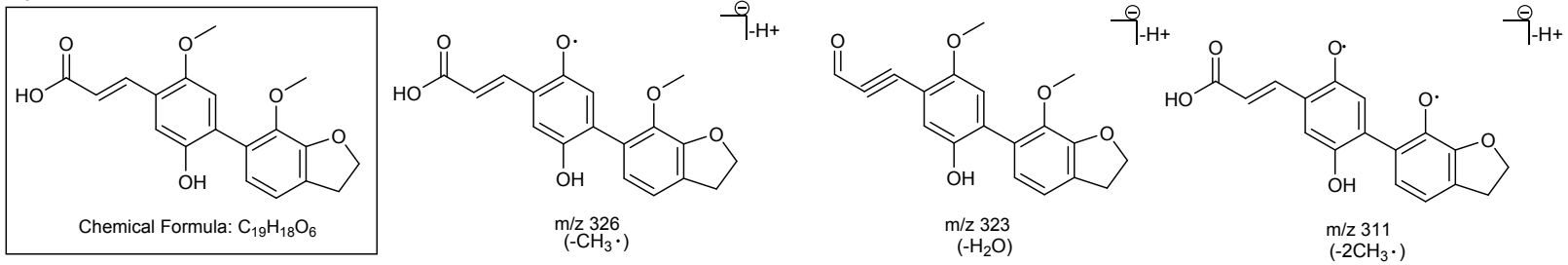
S1 Table 3 (*continued*). Proposed MS/MS fragments (*continued*).

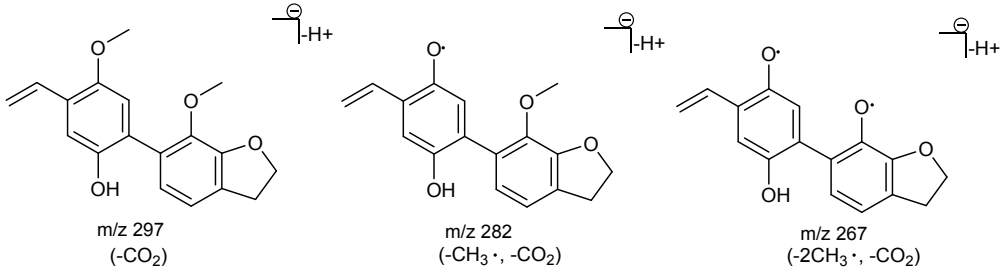


15

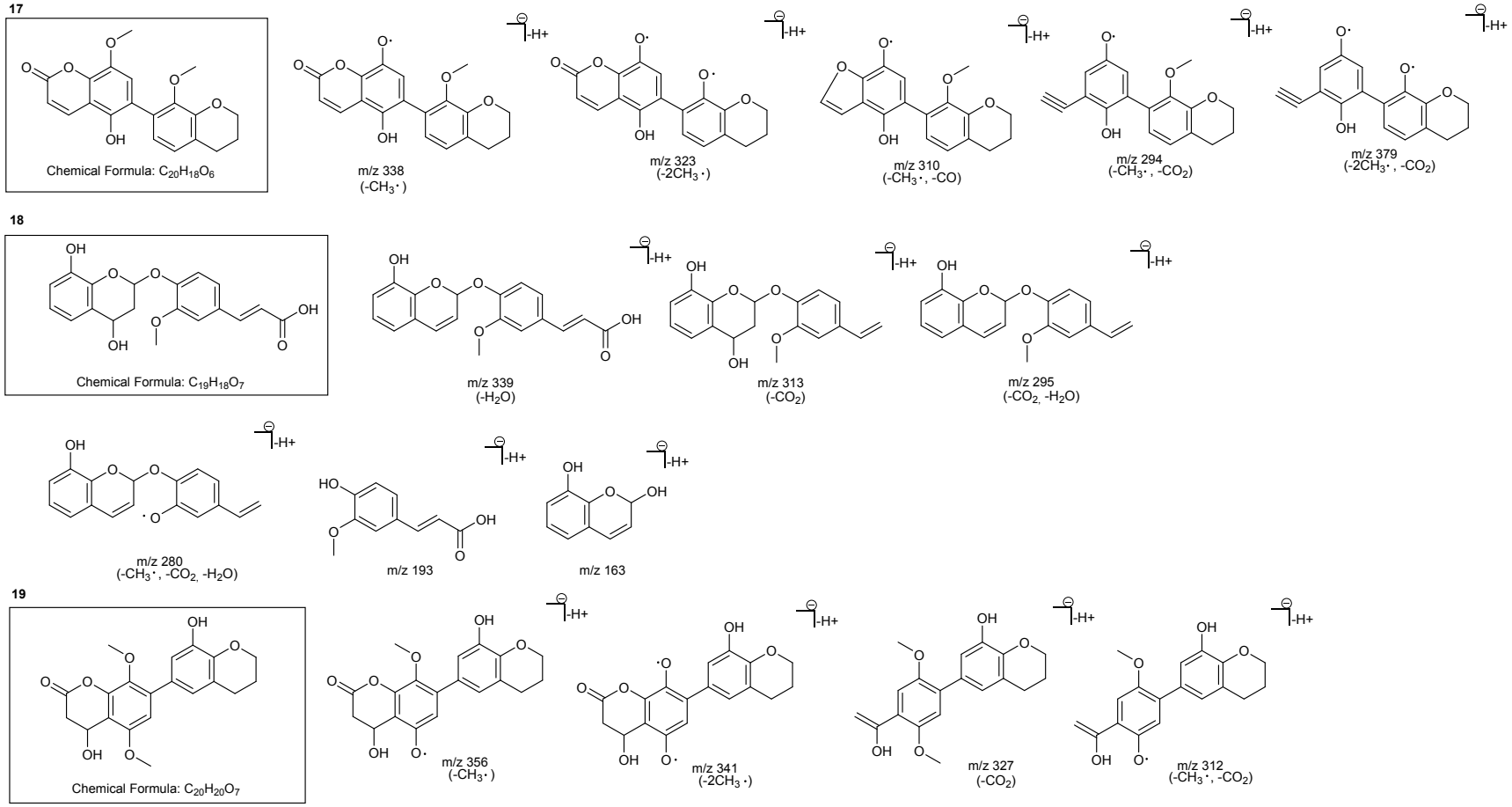


16

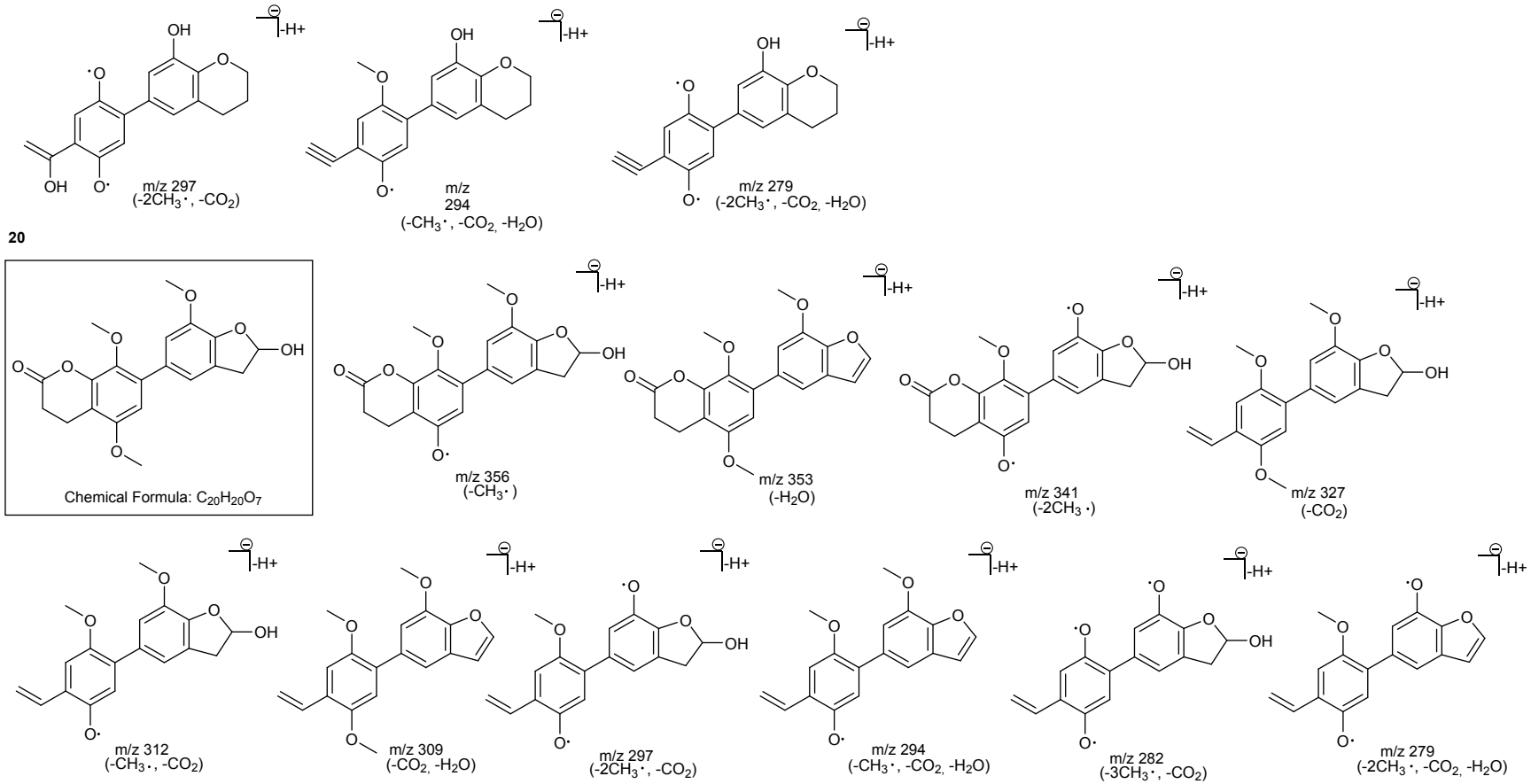




S1 Table 3 (*continued*). Proposed MS/MS fragments (*continued*).

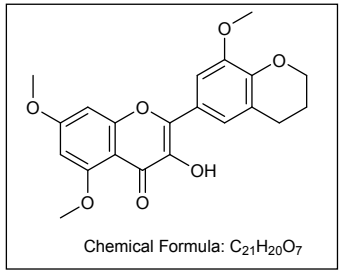


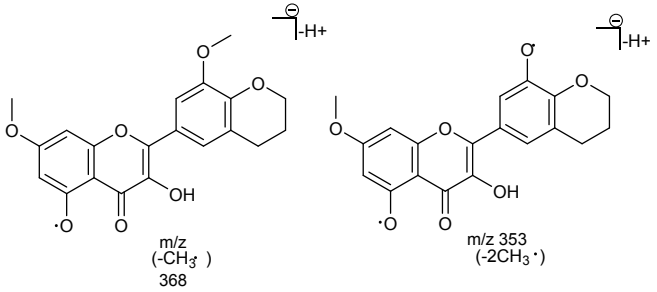
S1 Table 3. (*continued*). Proposed MS/MS fragments (*continued*).



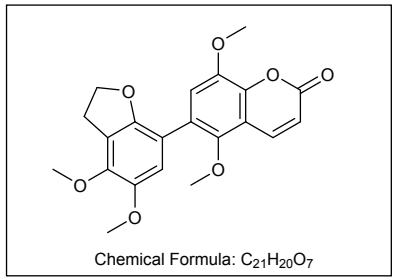
S1 Table 3 (*continued*). Proposed MS/MS fragments (*continued*).

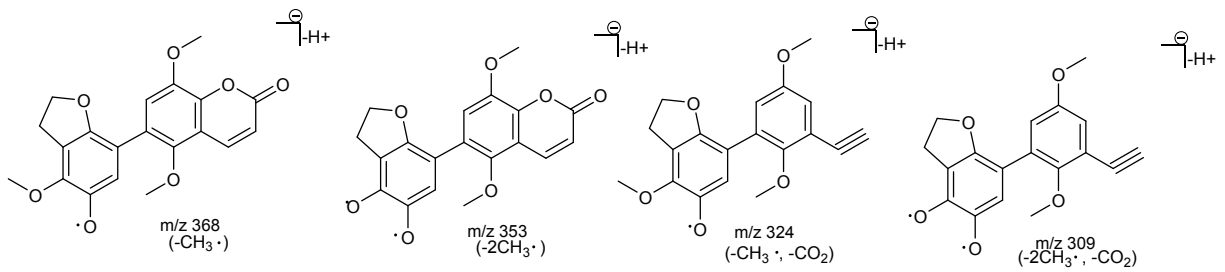
21



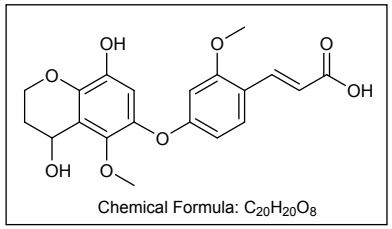


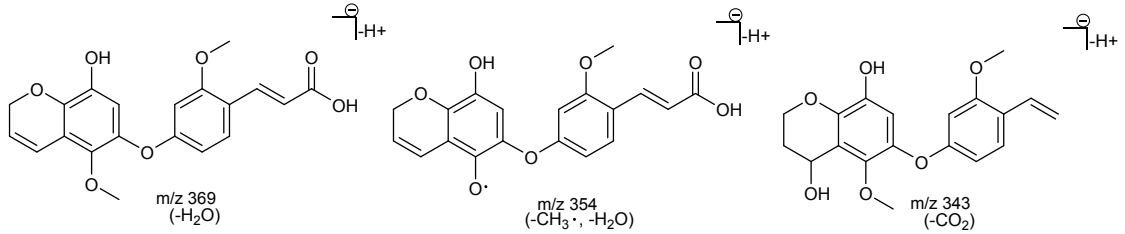
22

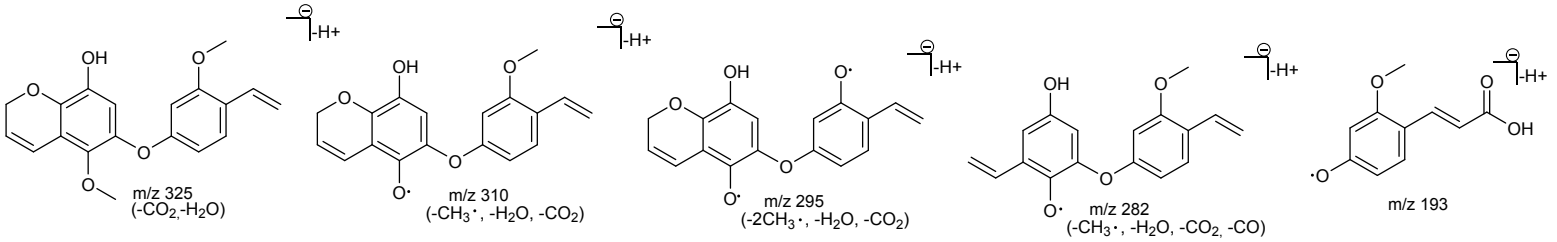




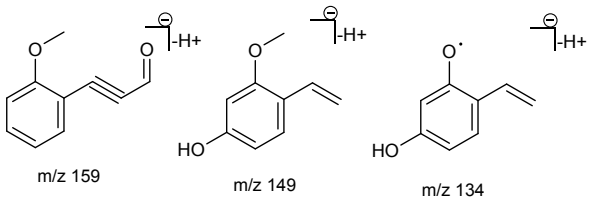
23





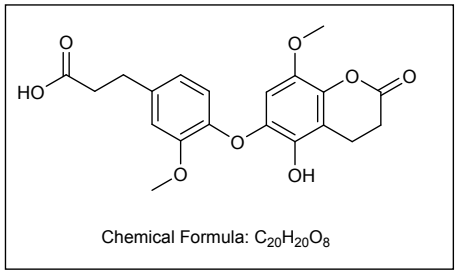


S1 Table 3 (*continued*). Proposed MS/MS fragments.

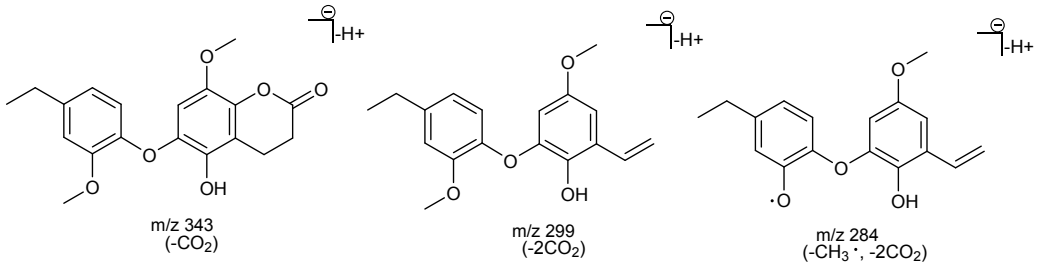


m/z 159 m/z 149 m/z 134

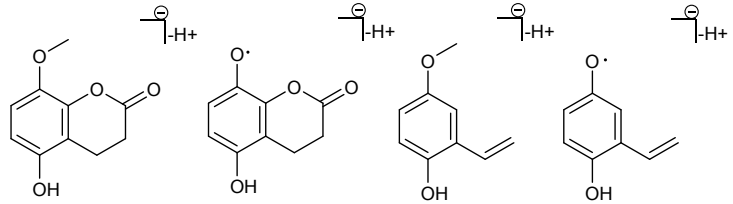
24



Chemical Formula: $C_{20}H_{20}O_8$

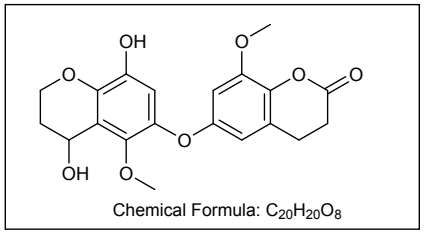


m/z 343 ($-CO_2$) m/z 299 ($-2CO_2$) m/z 284 ($-CH_3^{\cdot}$, $-2CO_2$)

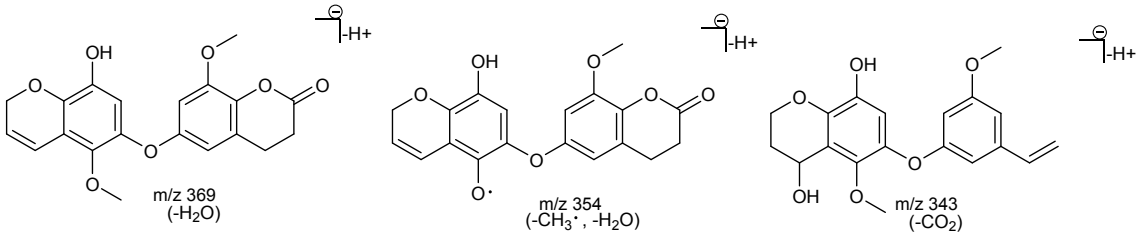


m/z 193 m/z 178 m/z 149 m/z 134

25

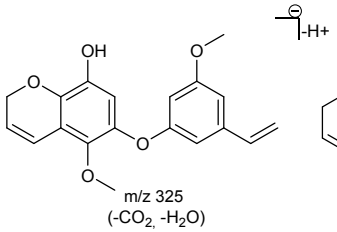


Chemical Formula: $C_{20}H_{20}O_8$

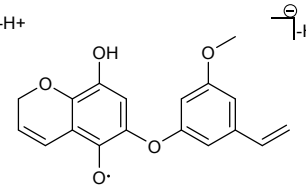


m/z 369 ($-H_2O$) m/z 354 ($-CH_3^{\cdot}$, $-H_2O$) m/z 343 ($-CO_2$)

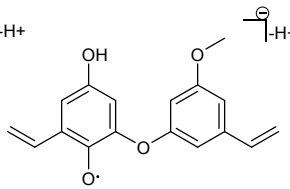
S1 Table 3 (*continued*). Proposed MS/MS fragments.



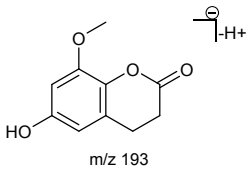
m/z 325
($-CO_2$, $-H_2O$)



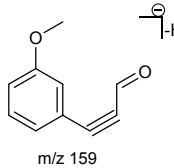
m/z 310
($-CH_3\cdot$, $-H_2O$, $-CO_2$)



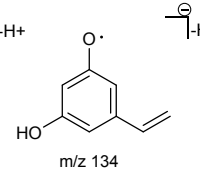
m/z 282
($-CH_3\cdot$, $-H_2O$, $-CO_2$, $-CO$)



m/z 193

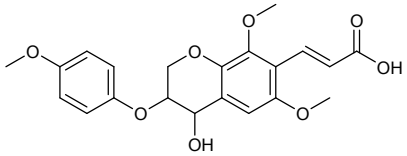


m/z 159

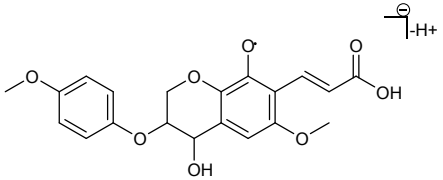


m/z 134

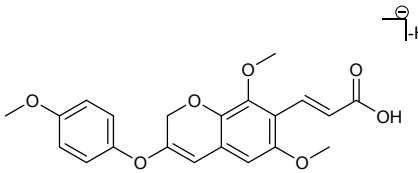
26



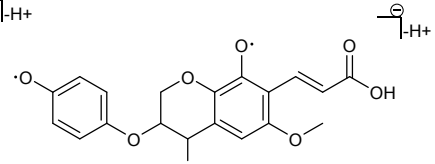
Chemical Formula: $C_{21}H_{22}O_8$



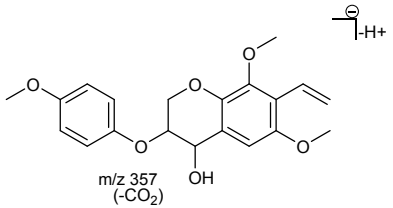
m/z 386
($-CH_3\cdot$)



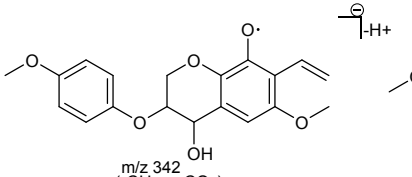
m/z 383
($-H_2O$)



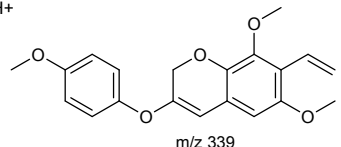
m/z 371
($-2CH_3\cdot$)



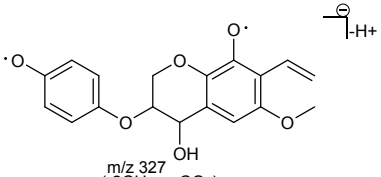
m/z 357
($-CO_2$)



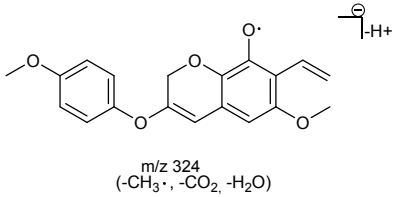
m/z 342
($-CH_3\cdot$, $-CO_2$)



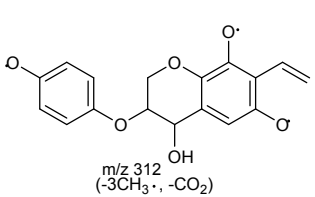
m/z 339
($-H_2O$, $-CO_2$)



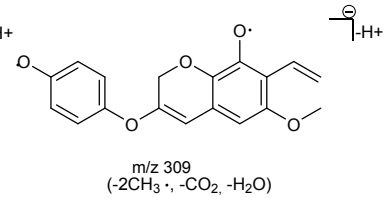
m/z 327
($-2CH_3\cdot$, $-CO_2$)



m/z 324
($-CH_3\cdot$, $-CO_2$, $-H_2O$)



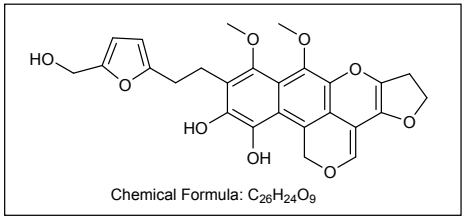
m/z 312
($-3CH_3\cdot$, $-CO_2$)

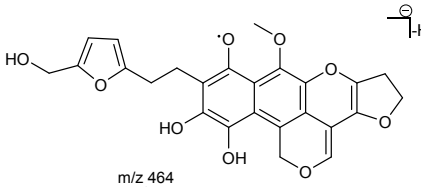


m/z 309
($-2CH_3\cdot$, $-CO_2$, $-H_2O$)

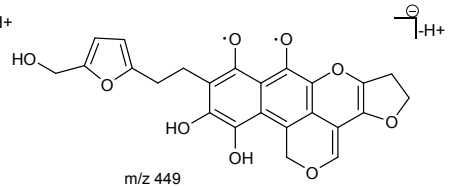
S1 Table 3 (*continued*). Proposed MS/MS fragments.

27

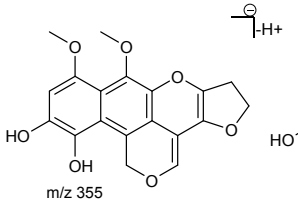




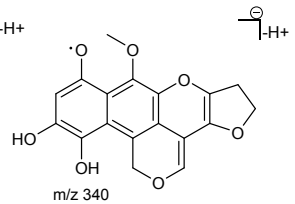
m/z 464
(-CH_3·)



m/z 449
(-$2CH_3$·)

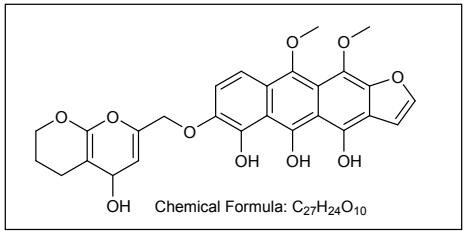


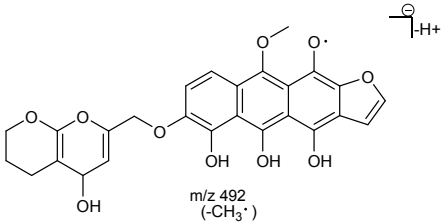
m/z 355



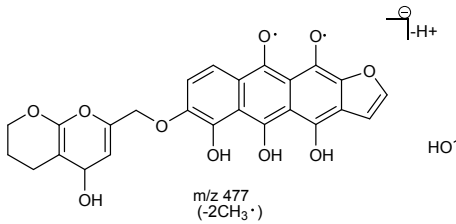
m/z 340

28

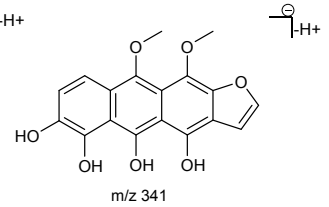




m/z 492
(-CH_3·)

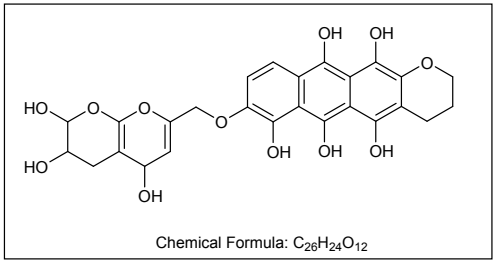


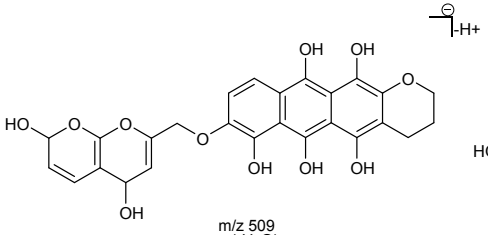
m/z 477
(-$2CH_3$·)



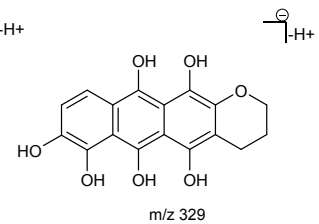
m/z 341

29

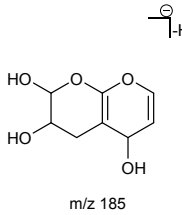




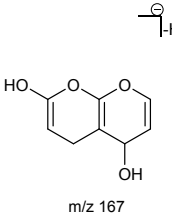
m/z 509
(-H_2O)



m/z 329



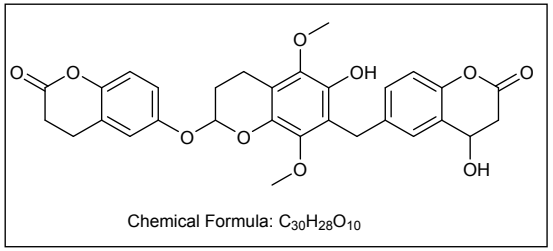
m/z 185

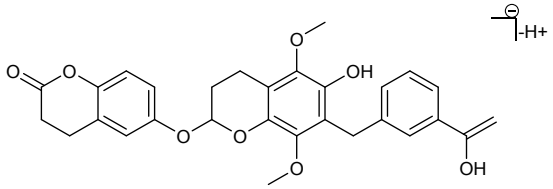


m/z 167

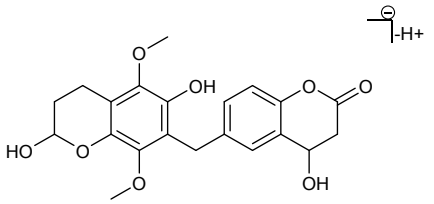
S1 Table 3 (*continued*). Proposed MS/MS fragments.

30





m/z 503
($-CO_2$)



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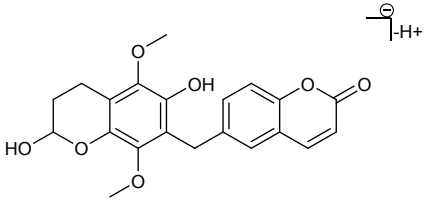

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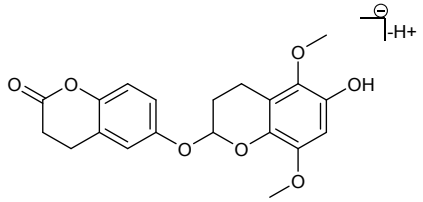
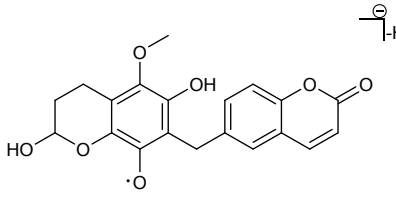
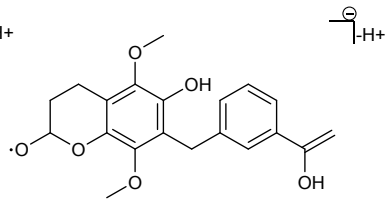


m/z 371



m/z 368



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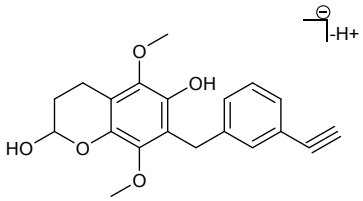
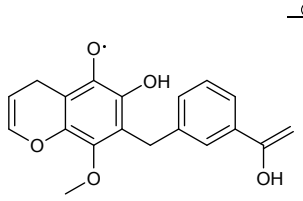
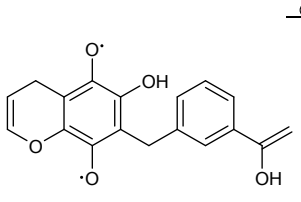


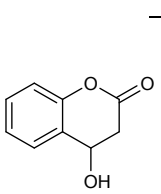
m/z 339



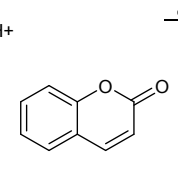
m/z 324



m/z 309



m/z 163



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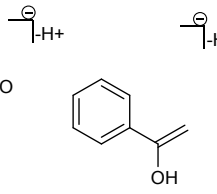
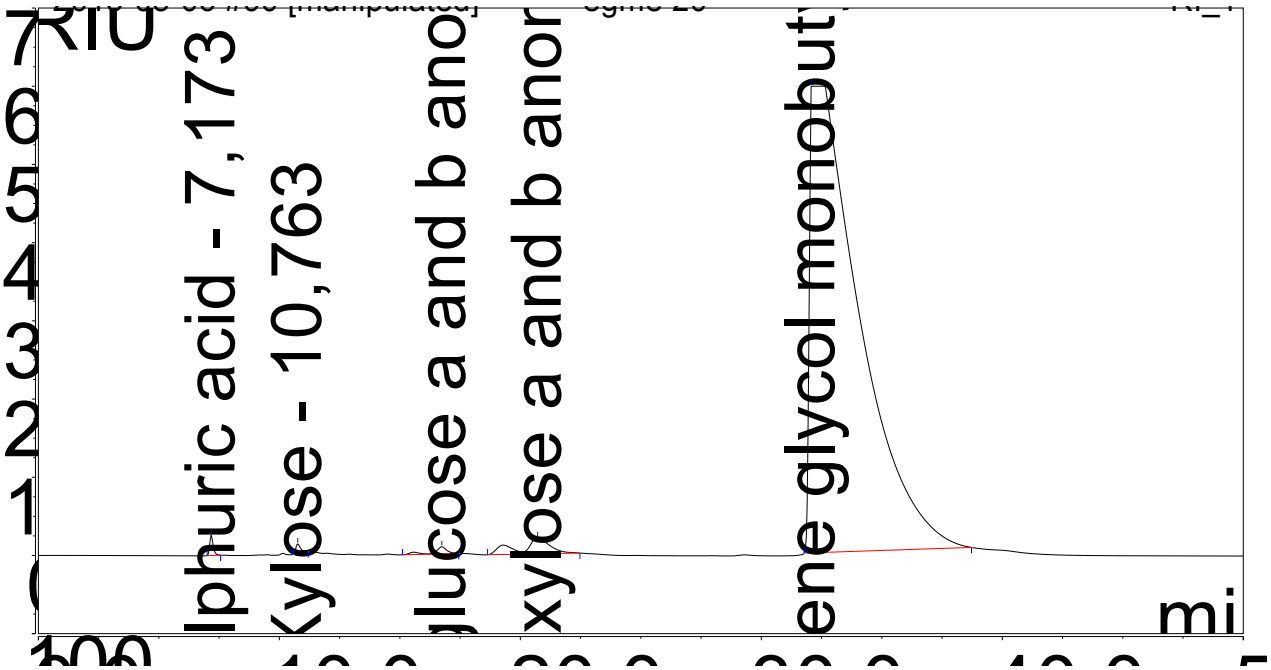


m/z 119

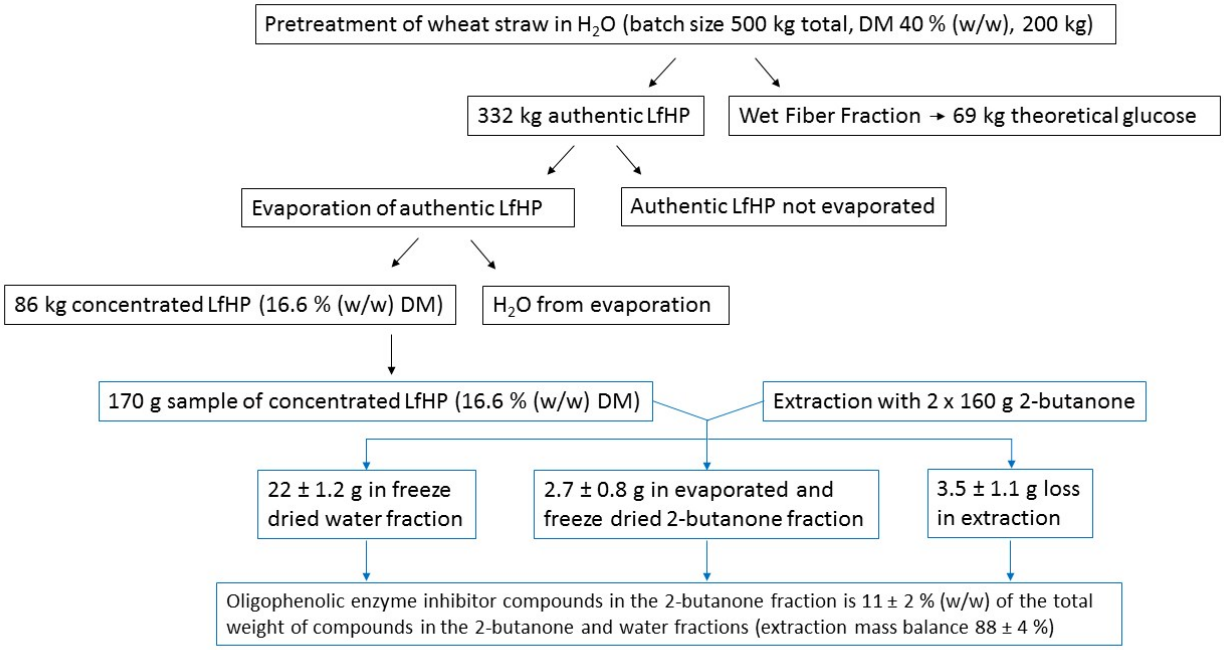
S1 Table 4. Compounds in the water fraction from 2-butanone extraction and 2-butanone extraction in the retention time region from 3.7-5.5 minutes.

Retention time (min)	Ions, *m/z* [M- H^+]$^-$ (relative intensity)	Fraction	Compounds
3.95	455.14203 (3), 587.18378 (12), 719.22626 (14), 851.26862 (9), 983.31140 (6),1115.35315 (2)	Water fraction	Pentoses + 1 acetyl, DP 3, DP 4, DP 5, DP 6, DP 7, DP 8.
3.95	455.14233 (4), 587.18451 (6)	2-butanone fraction	Pentoses + 1 acetyl, DP 3, DP 4.
4.14	629.19318 (16), 761.23517 (29), 893.27692 (20), 1025.31921 (12), 1157.36060 (5), 1199.37134 (8), 1331.41345 (6), 1463.45447 (4)	Water fraction	Pentoses + 2 acetyl, DP 4, DP 5, DP 6, DP 7, DP 8. Pentoses + 3 acetyl, DP 8, DP 9, DP 10.
4.14	629.19604 (0.1)	2-butanone fraction	Pentose + 2 acetyl, DP 4
4.54	803.24573 (4), 935.28717 (4), 1249.38831 (7), 1381.43054 (3)	Water fraction	Pentoses + 3 acetyl, DP 5, DP 6. Feruloyl substituted pentoses, DP 8, DP 9.
4.68	985.30377 (20), 1117.34460 (4), 1249.38843 (4)	Water fraction	Feruloyl substituted pentoses, DP 6, DP 7, DP 8.
4.88	691.20862 (24), 721.21936 (32), 853.26135 (100), 1027.31409 (8), 1159.35547 (12), 1291.39734 (4)	Water fraction	Cumaroyl substituted pentose DP 4. Feruloyl substituted pentose DP 4, DP 5. Feruloyl + 1 acetyl substituted pentose DP 6, DP 7, DP 8.
4.98	559.16693 (6), 721.21985 (5)	2-butanone fraction	Cumaroyl substituted pentose DP 3. Feruloyl substituted pentose DP 4.
5.00	721.21936 (100), 1027.31445 (9)	Water fraction	Feruloyl substituted pentose DP 4. Feruloyl + 1 acetyl substituted pentose DP 7.

Retention time (min)	Ions, *m/z* [M- H^+]$^-$ (relative intensity)	Fraction	Compounds
5.05	589.17706 (100), 721.21906 (16), 853. 26074 (7), 1117.34448 (10) 895.27173 (16), 1027.31494 (8), 1159.35559 (5), 1291.39893 (4), 1423.43689 (3)	Water fraction	Feruloyl substituted pentoses, DP 3, DP 4, DP 5, DP 7. Feruloyl + 1 acetyl substituted pentoses, DP 5, DP 6, DP 7, DP 8, DP 9.
5.05	589.17737 (30)	2-butanone fraction	Feruloyl substituted pentose DP 3.
5.27	325.09247 (100), 559.16699 (17), 763.23035 (6),	2-butanone fraction	Feruloyl substituted pentose DP 1. Cumaroyl substituted pentose DP 3. Feruloyl + 1 acetyl substituted pentose DP 4.
5.47	295.08221 (15), 457.13538 (8)	2-butanone fraction	Cumaroyl substituted pentose DP 1. Feruloyl substituted pentose DP 2.



S1 Figure 2. HPLC chromatogram with refractive index detection, anomeric protection.



S1 Figure 3. Flow chart of the pilot plant pretreatment of wheat straw and the laboratory extraction of LfHP (Liquid from Hydrothermal Pretreatment). The dry matter (DM) contents in LfHP are the w/w % of dissolved compounds (as the oligophenolic compounds and xylooligosaccharides) in the LfHP (H_2O). The LfHP is evaporated in order to obtain a more concentrated solution for further pilot plant processing. Subsequent to pilot plant evaporation a representative sample (170 g) was taken from the concentrated LfHP fraction to conduct laboratory evaluation of compounds in the LfHP. Boxes in blue color are laboratory conducted experiments: As indicated, the dry matter content of the 170 g sample constituted 16.6 % by weight (as determined from drying via evaporation of the liquid), and this amount of dry matter was equivalent to 28.22 g of material which was distributed into two fractions after extraction with 2 x 160 g 2-butanone as follows: 22 g in the freeze dried aqueous fraction; 2.7 g in the 2-butanone fraction, and with a loss of 3.5 g dry matter in total. The 22 g in the freeze dried aqueous fraction of the sample was equivalent to approximately 78% of the dry matter in the concentrated LfHP, whereas the 2.7 g in the 2-butanone fraction constituted just above 9.5% of the dry matter in the concentrated LfHP.