

Supplementary material

Table S1 – Antimicrobial activity of esculin oligomer fractions from reaction A with esculin at 2 g/L in acetate buffer (0.1 M, pH 5), expressed as colony forming units per mL present in the cell culture after 24 h of incubation with test compounds

	<i>E. coli</i>	<i>Salmonella</i>
	CFU/mL	
Negative control	0	
Positive control	$1.79 \cdot 10^9$	$3.35 \cdot 10^{10}$
Pellet		
7.5 g/L	$1.15 \cdot 10^9$	$2.67 \cdot 10^{10}$
5 g/L	$1.66 \cdot 10^9$	$6.00 \cdot 10^{10}$
2.5 g/L	$4.00 \cdot 10^9$	$5.94 \cdot 10^{10}$
Oligomers >10 kDa		
7.5 g/L	$2.63 \cdot 10^9$	$1.73 \cdot 10^{10}$
5 g/L	$1.94 \cdot 10^9$	$1.54 \cdot 10^{10}$
2.5 g/L	$4.00 \cdot 10^9$	$2.40 \cdot 10^{10}$

Table S2 –MALDI-TOF results of the supernatant from reaction A and suggested compounds, detected either as the protonated compound and/or the Na-adduct.

Observed mass (Da)	Intensity	Suggested compound	Theor. mass (Da)	Δ mass (Da)
362.88	594.52	(E+Na) ⁺	363.26	
701.14	1329.08	(2E+Na) ⁺	701.53	338.26
1039.27	2315.97	(3E+Na) ⁺	1039.79	338.13
1377.37	3899.59	(4E+Na) ⁺	1378.06	338.10
1715.45	1149.58	(5E+Na) ⁺	1716.33	338.08
2053.52	437.9	(6E+Na) ⁺	2054.59	338.08
2391.61	131.11	(7E+Na) ⁺	2392.86	338.09
2730.66	64.13	(8E+Na) ⁺	2731.13	339.05
3069.73	33.83	(9E+Na) ⁺	3069.39	339.07
340.90	570.24	E ⁺	341.28	
679.06	276.13	2E ⁺	679.55	338.16
1015.26	88.2	3E ⁺	1017.81	336.21
1355.30	62.3	4E ⁺	1356.08	340.04
1693.46	50.0	5E ⁺	1694.35	338.16
539.00	218.6	(2E-G+Na) ⁺	539.39	
877.19	163.48	(3E-G+Na) ⁺	877.65	338.19
1215.29	236.74	(4E-G+Na) ⁺	1215.92	338.10
1553.37	165.95	(5E-G+Na) ⁺	1554.19	338.09
1891.45	102.47	(6E-G+Na) ⁺	1892.45	338.08
2231.54	60.18	(7E-G+Na) ⁺	2230.72	340.09
517.00	230.84	(2E-G) ⁺	517.41	
855.20	220.67	(3E-G) ⁺	855.67	338.21
1193.29	159.95	(4E-G) ⁺	1193.94	338.09
1531.39	178.52	(5E-G) ⁺	1532.21	338.10
1869.48	92.78	(6E-G) ⁺	1870.47	338.09
2207.54	55.99	(7E-G) ⁺	2208.74	338.06
354.86	854.67	2e ⁺	355.28	
530.97	1315.9	3e ⁺	531.40	176.10
707.05	1275.64	4e ⁺	707.53	176.09
883.13	541.63	5e ⁺	883.65	176.07
1059.15	342.17	6e ⁺	1059.78	176.02
1235.18	159.59	7e ⁺	1235.90	176.03
1411.23	74.56	8e ⁺	1412.03	176.05
1587.38	59.76	9e ⁺	1588.16	176.15
1763.39	46.38	10e ⁺	1764.28	176.01

Table S2 (cont) –MALDI-TOF results of the supernatant from reaction A and suggested compounds, detected either as the protonated compound and/or the Na-adduct.

Observed mass (Da)	Intensity	Suggested compound	Theor. mass (Da)	Δ mass (Da)
525.00	134.36	(E+G+Na) ⁺	525.40	
863.17	66.41	(2E+G+Na) ⁺	863.67	338.17
502.96	153.23	(E+G) ⁺	503.42	
841.17	82.07	(2E+G) ⁺	841.69	338.21
1179.29	62.16	(3E+G) ⁺	1179.96	338.12
1517.40	60.55	(4E+G) ⁺	1518.22	338.11
1855.46	49.72	(5E+G) ⁺	1856.49	338.06

Table S3 –MALDI-TOF results of the pellet from reaction A and suggested compounds, detected either as the protonated compound and/or the Na-adduct.

Observed mass	Intensity	Suggested compound	Theoretical mass	Δ mass (Da)
362.85	68.62	(E+Na) ⁺	363.26	
701.07	211.72	(2E+Na) ⁺	701.53	338.22
1039.17	238.2	(3E+Na) ⁺	1039.79	338.10
1377.24	1635.12	(4E+Na) ⁺	1378.06	338.07
1715.29	231.29	(5E+Na) ⁺	1716.33	338.05
2053.35	121.29	(6E+Na) ⁺	2054.59	338.06
340.85	169.46	E ⁺	341.28	
679.00	208.74	2E ⁺	679.55	338.14
538.85	192.06	(2E-G+Na) ⁺	539.39	
1215.18	88.96	(4E-G+Na) ⁺	1215.92	
1553.22	39.17	(5E-G+Na) ⁺	1554.19	338.04
516.93	122.85	(2E-G) ⁺	517.41	
855.08	102.87	(3E-G) ⁺	855.67	338.15
1193.19	112.27	(4E-G) ⁺	1193.94	338.11
1531.244	79.25	(5E-G) ⁺	1532.206	338.06
354.82	1152.75	2e ⁺	355.28	
530.92	1268.18	3e ⁺	531.40	176.10
707.00	3028.43	4e ⁺	707.53	176.08
883.06	770.27	5e ⁺	883.65	176.06
1059.07	762.8	6e ⁺	1059.78	176.01
1235.07	174	7e ⁺	1235.90	176.01
1411.13	259.67	8e ⁺	1412.03	176.05
1587.10	54.64	9e ⁺	1588.16	175.97
502.89	121.52	(E+G) ⁺	503.42	

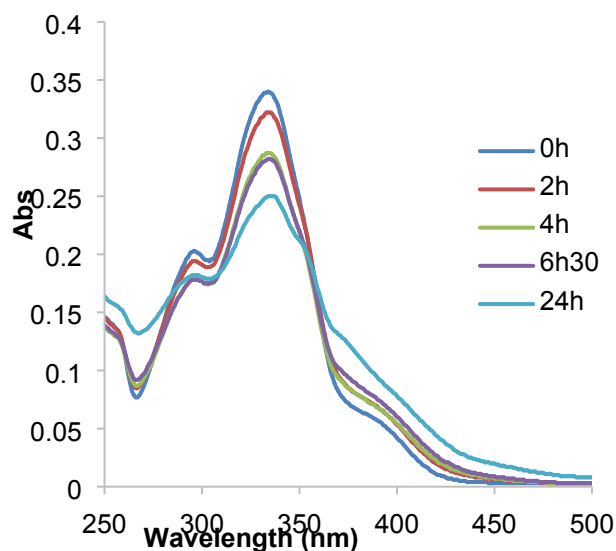


Figure S1. UV-vis spectrum of the laccase-transformation products from reaction B: esculin at 5 g/L in 30:70 (v/v) ethanol/acetate buffer

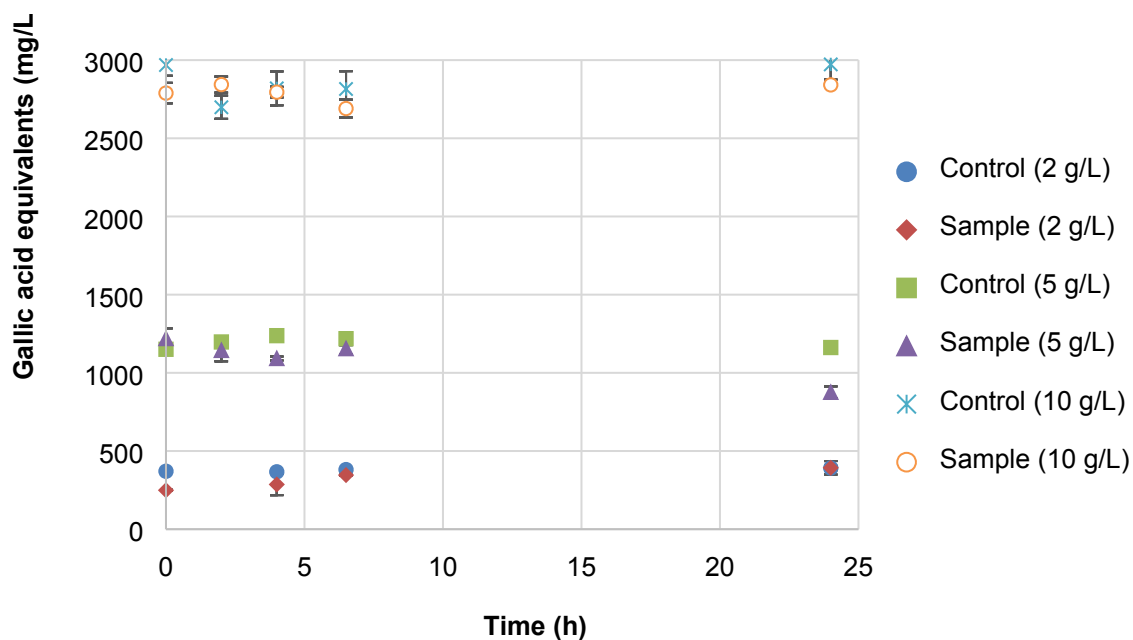


Figure S2. Evaluation of the Total Phenolic Compounds (TPC) content of the supernatants from reactions A (2 g/L esculin in acetate buffer), B (5 g/L esculin in ethanol:acetate buffer, 30:30) and C (10 g/L esculin in ethanol:acetate buffer, 50:50)

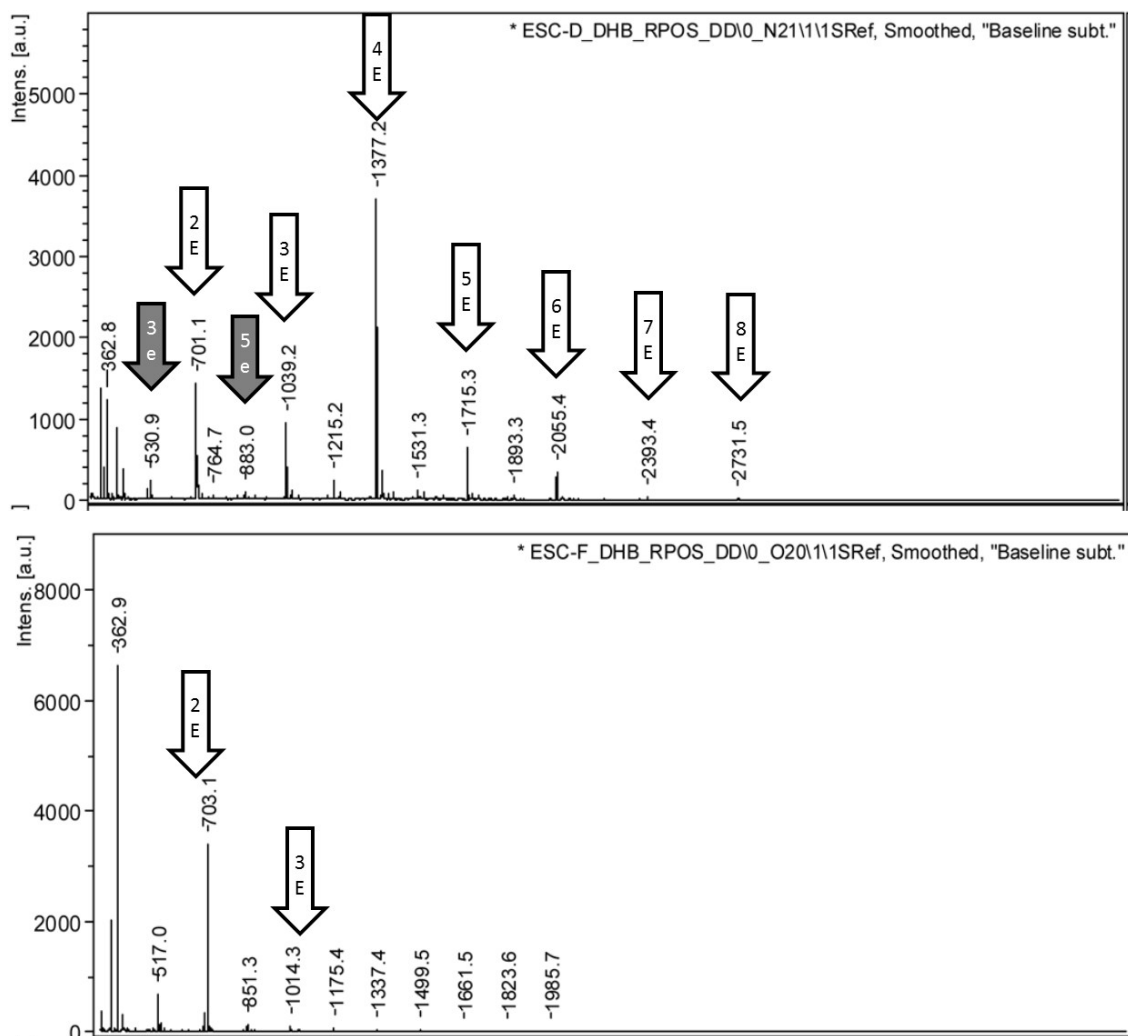


Figure S3. MALDI-TOF analysis of supernatants from reactions B (upper figure) and C (lower figure) (esculin at 5 and 10 g/L in 30% and 50% ethanol, respectively). XE or Xe means oligomer of esculetin or esculin, respectively, with a DP of X

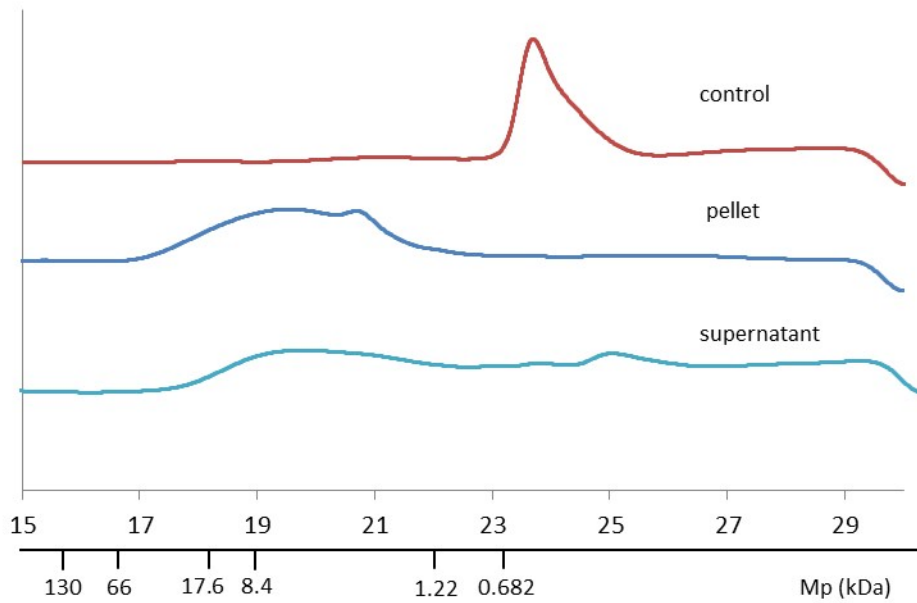


Figure S4 –Relative masses of polymers from reaction A were evaluated by size exclusion chromatography(HPLC-HP1100 with RI detector, Agilent POLARGEL-L (100-60000) 300 x 7.5 mm and 8 μ m column, 50 $^{\circ}$ C). Dimethylformamide (DMF) with 1% LiBr was used as a mobile phase (0.4 mL/min). Control contained esculin in acetate buffer. Molecular mass calibration was obtained using standards of polystyrene (Sigma-Aldrich)