Supporting Information

Polymerized Luteolin Nanoparticles: Synthesis, Structure Elucidation and Anti-Inflammatory Activity

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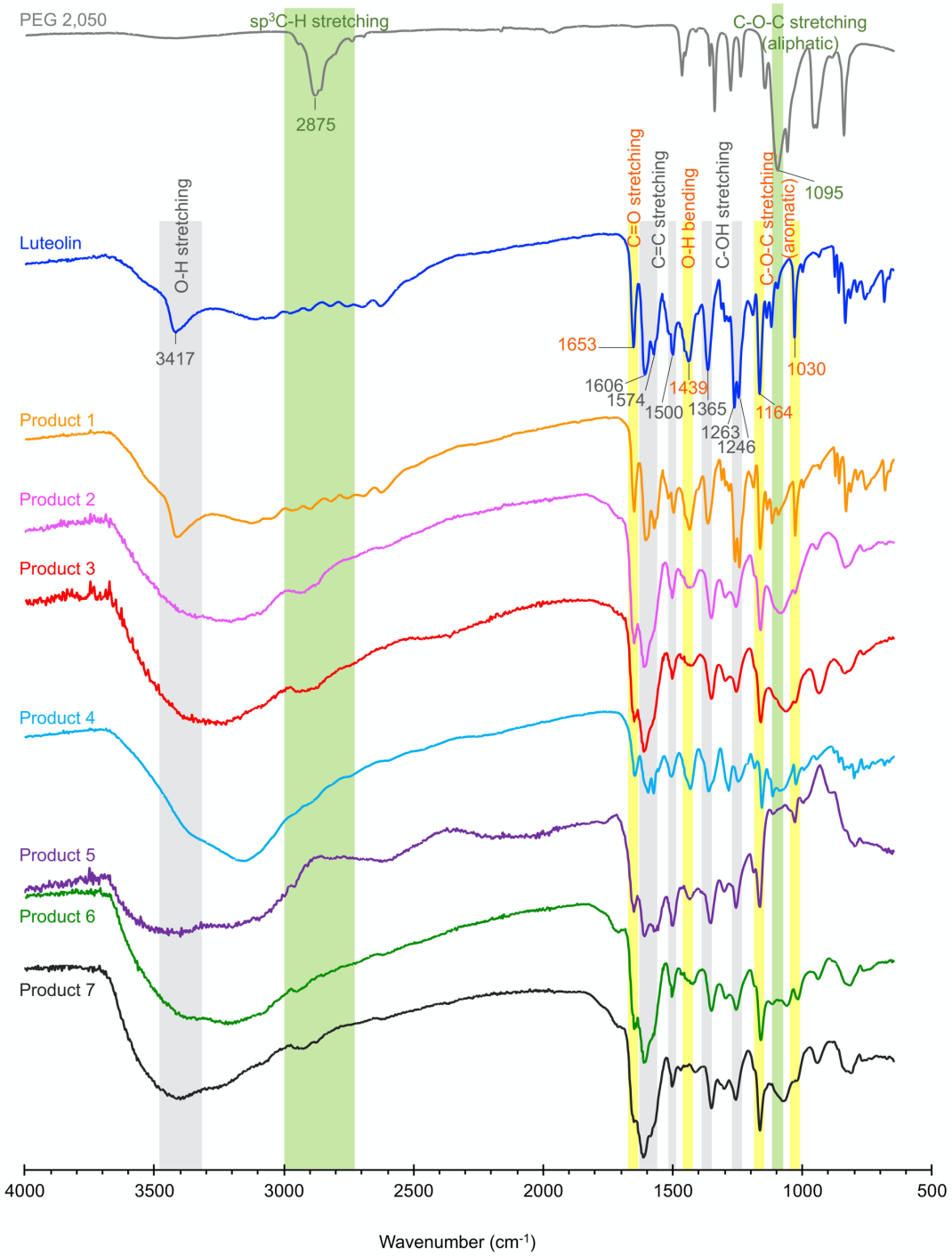

Figure S1. IR spectra of luteolin, and products prepared under various conditions: no HRP | H_2O_2 | PEG 2050 (Product 1); 1.7% HRP | H_2O_2 | PEG 2050 (Product 2); 3.3% HRP | H_2O_2 | PEG 2050 (Product 3); 3.3% HRP | no H_2O_2 | PEG 2050 (Product 4); 3.3% HRP | H_2O_2 | no PEG (Product 5); 3.3% HRP | H_2O_2 | PEG 200 (Product 6); 3.3% HRP | H_2O_2 | PEG 35000 (Product 7).

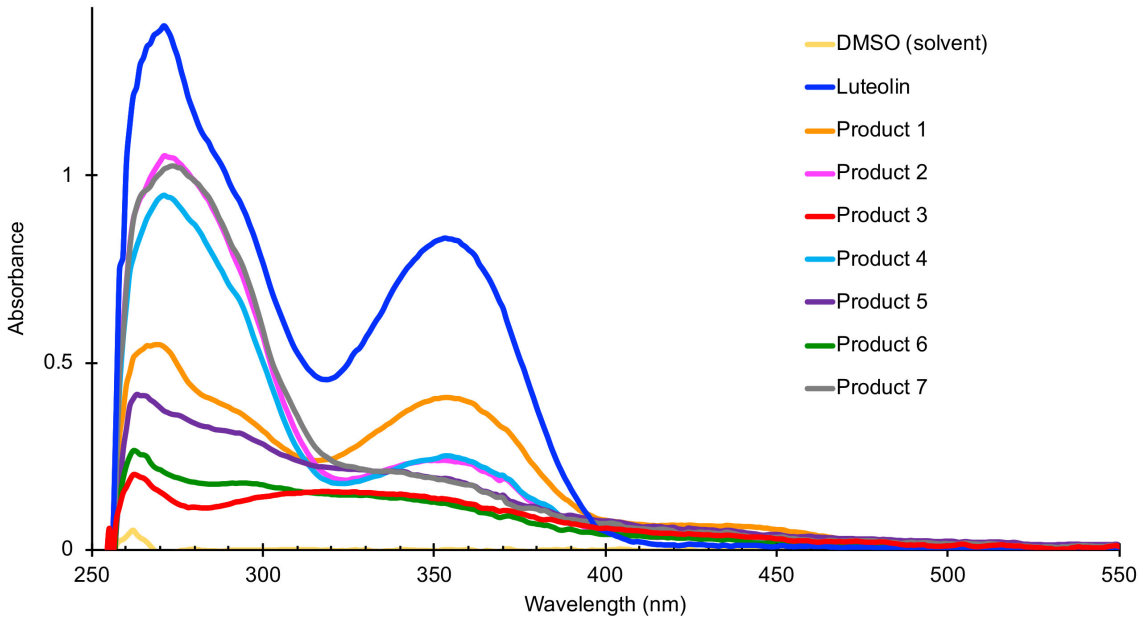

Figure S2. UV spectra (10 µg/mL, DMSO) of luteolin, and products prepared under various conditions: no HRP | H_2O_2 | PEG 2050 (Product 1); 1.7% HRP | H_2O_2 | PEG 2050 (Product 2); 3.3% HRP | H_2O_2 | PEG 2050 (Product 3); 3.3% HRP | no H_2O_2 | PEG 2050 (Product 4); 3.3% HRP | H_2O_2 | no PEG (Product 5); 3.3% HRP | H_2O_2 | PEG 200 (Product 6); 3.3% HRP | H_2O_2 | PEG 35000 (Product 7).

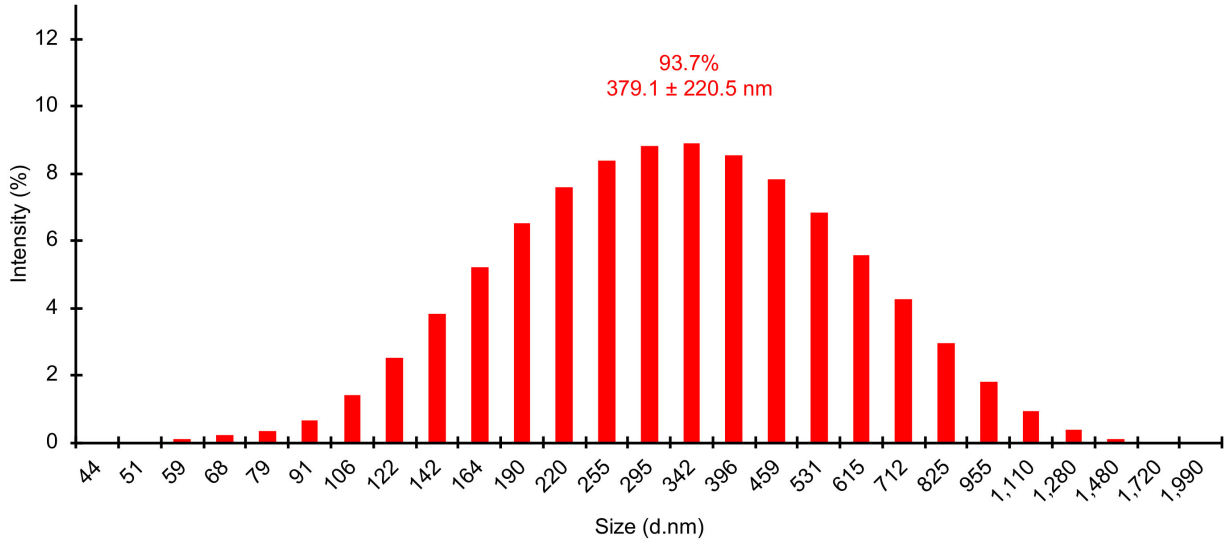

Figure S3. Particle size distribution of polyluteolin nanoparticles (Product 3) in water. Measurement was performed by dynamic light scattering technique. The graph represents average plot from three different measurements. Average size is 379.1 ± 220.5 nm and the polydispersity index is 0.338.

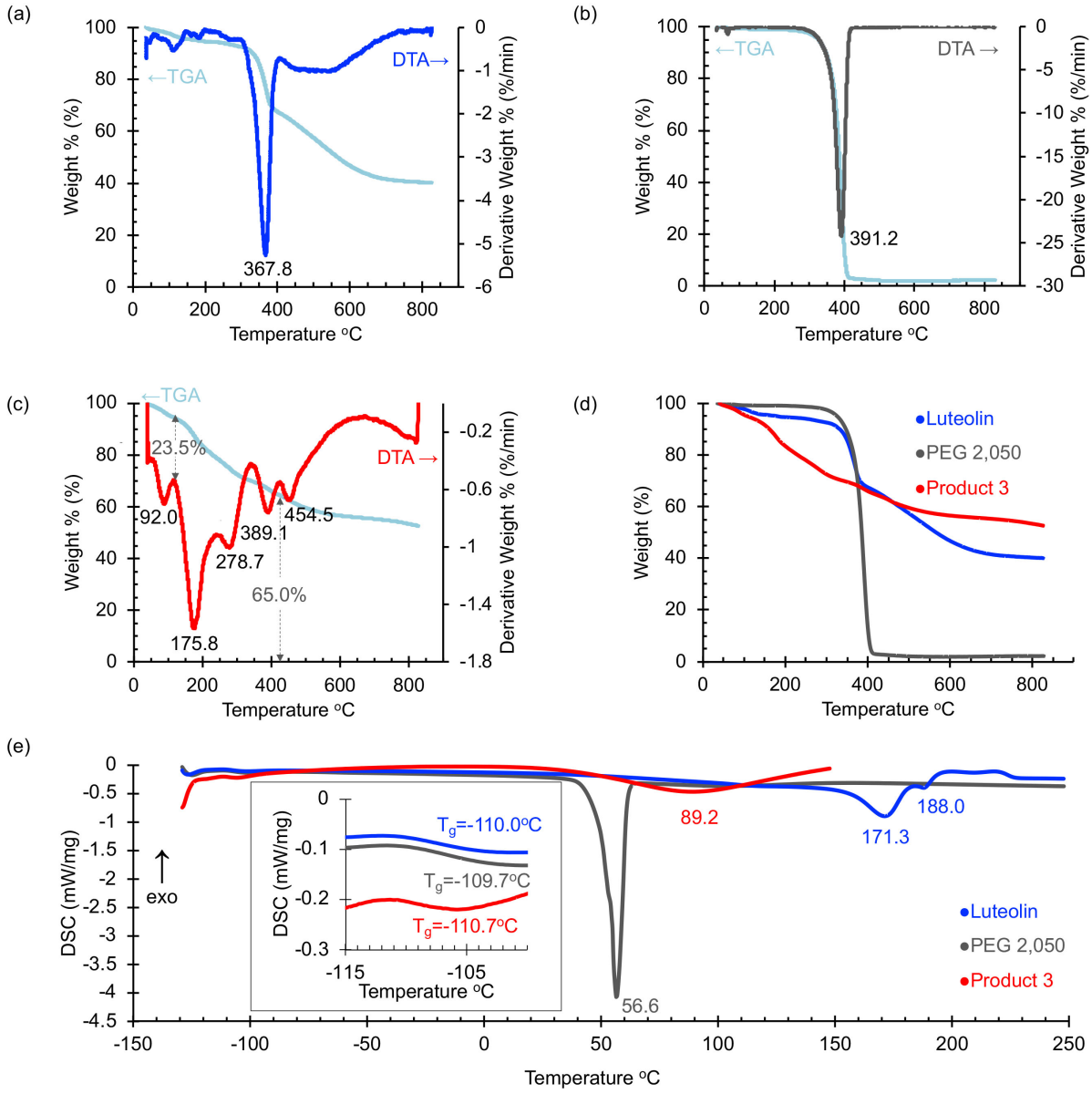

Figure S4. Thermal property of starting materials and Product 3: **TG-DTA curves** of luteolin (a), PEG 2050 (b), Product 3 (c); **TGA curves** of luteolin, PEG 2050, and Product 3 (d); **DSC curves** of luteolin, PEG 2050, and Product 3 (e).

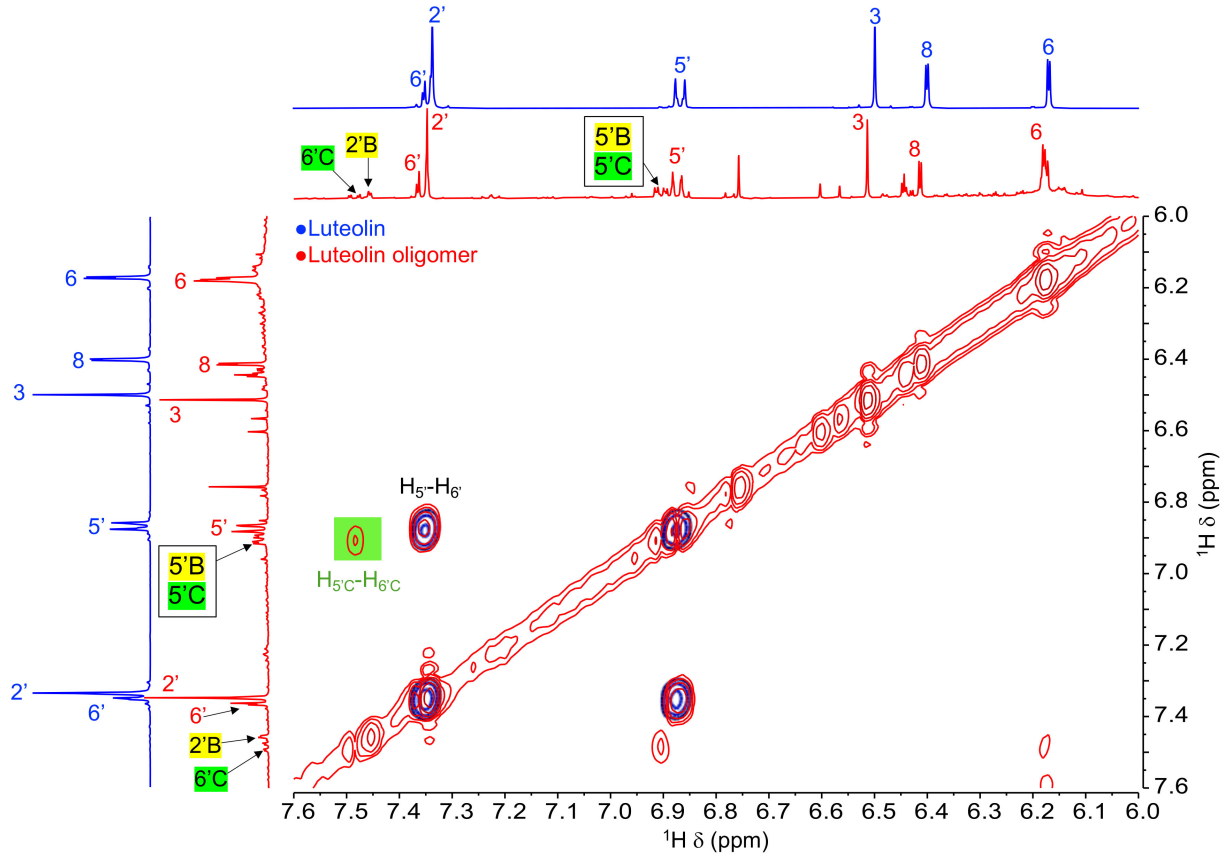

Figure S5. Overlay of ^{1}H-^{1}H COSY NMR correlations of luteolin and luteolin oligomers (MeOD-d4, 500 MHz).

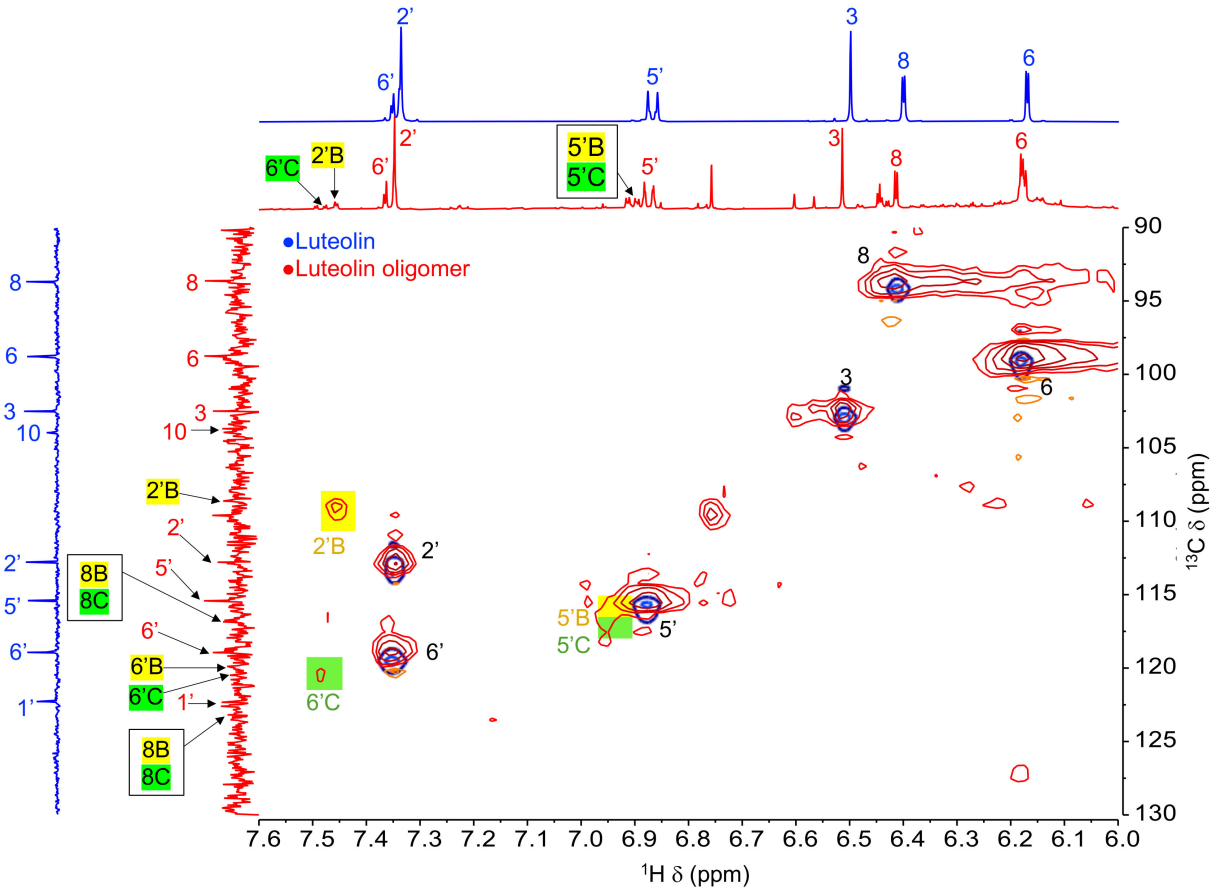

Figure S6. Overlay of ^{1}H-^{13}C HSQC NMR correlations of luteolin and luteolin oligomers (MeOD-d4, 500 MHz)

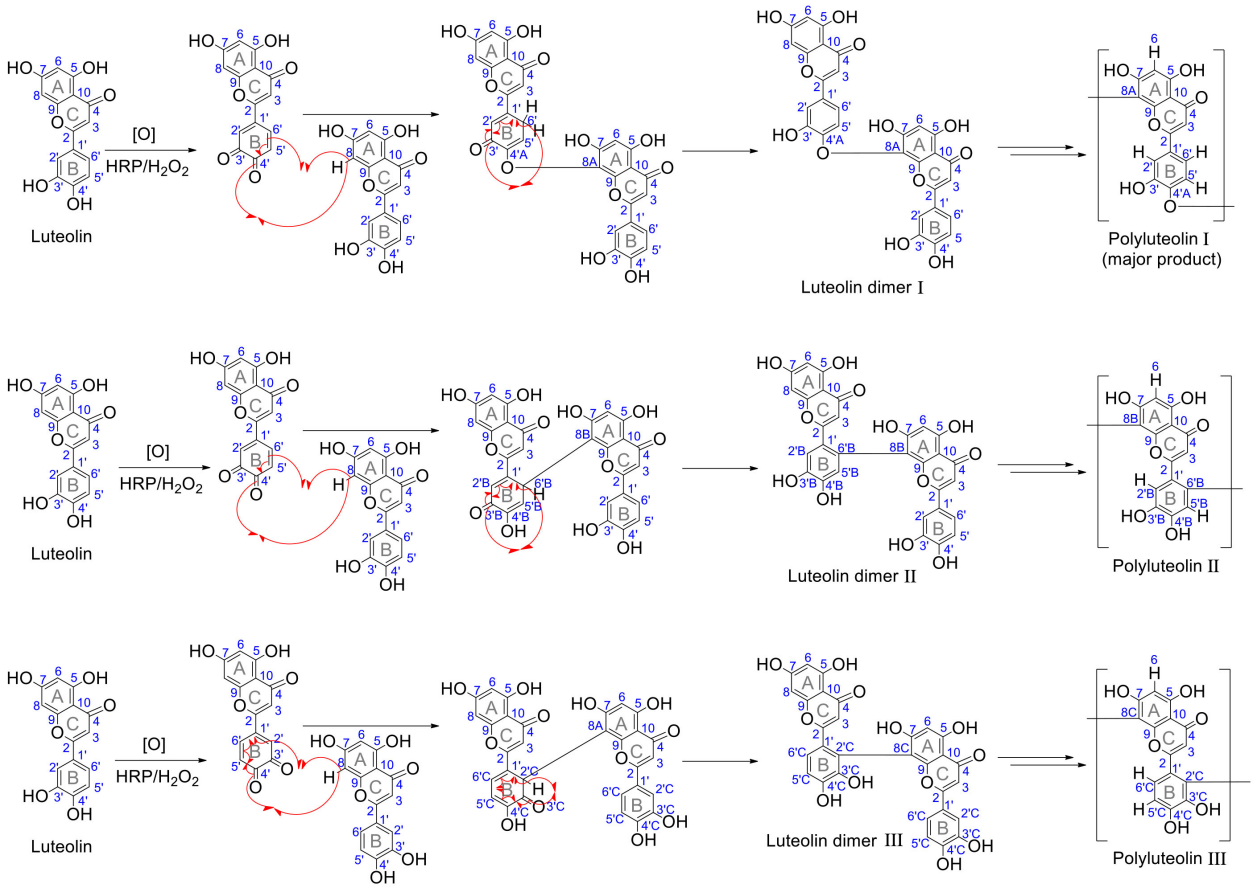

Figure S7. Three proposed structures and polymerization mechanism of the polymerized luteolin.

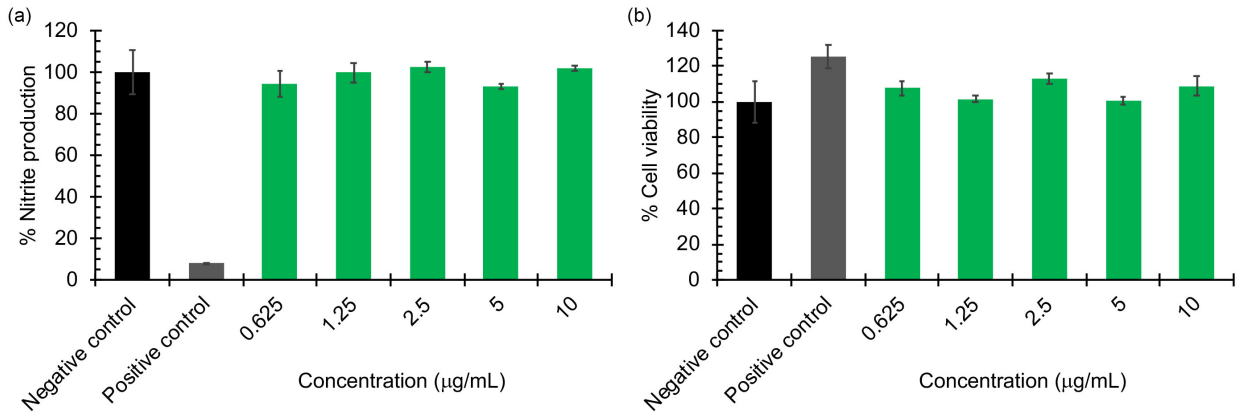

Figure S8. *In vitro* anti-inflammatory activity of PEG 2050: (a) %Nitrite production and (b) %Cell viability. Cells were incubated with various concentrations of PEG 2050 in aqueous suspension (10% relatively to the amounts of luteolin used in the reaction) for 1 h before stimulated with lipopolysaccharide (LPS) for 24 h. Nitrite levels in the culture supernatants were evaluated by the Griess reaction and cell viability was assessed using MTT assay. Cells treated with 1% water, IFN-γ and LPS in complete media was used as a negative control and cells treated with 1% water in complete media was used as a positive control. Each bar graph represents an average with the error bar showing standard deviation obtained from 3 independent experiments.

Table S1. ^{1}H and ^{13}C NMR chemical shift of luteolin oligomer II and III (MeOD-d4, 500 MHz).

Position	Luteolin oligomer II		Luteolin oligomer III	
	^{1}H δ (ppm), J (Hz)	^{13}C δ (ppm)	^{1}H δ (ppm), J (Hz)	^{13}C δ (ppm)
2	-	164.61	-	164.61
3	6.51 (1H, s)	102.50	6.51 (1H, s)	102.50
4	-	182.23	-	182.23
5	-	161.94	-	161.94
6	6.17 (1H, m)	98.74	6.17 (1H, m)	98.74
7	-	165.01	-	165.01
8	6.41 (1H, d, 2.3)	93.60	6.41 (1H, d, 2.3)	93.60
8B	-	123.12	-	-
8C	-	-	-	123.12
9	-	157.40	-	157.40
10	-	103.66	-	103.66
1’	-	122.40	-	122.40
2’	7.35 (1H, m)	112.73	7.35 (1H, m)	112.73
2’B	7.45 (1H, s) and 7.46 (1H, s)*	108.77	-	-
2’C	-	-	-	N/A
3’	-	145.85	-	145.85
3’B	-	148.34	-	-
3’C	-	-	-	148.34
4’	-	149.65	-	149.65
4’B	-	150.77	-	-
4’C	-	-	-	150.77
5’	6.87 (1H, dd, 8.9, 2.0)	115.38	6.87 (1H, dd, 8.9, 2.0)	115.38
5’B	6.90 (1H, s) and 6.91 (1H, s)**	116.58	-	-
5’C	-	-	6.90 (1H, d, 8.59) and 6.91 (1H, d, 8.30)***	116.58
6’	7.36 (1H, m)	118.81	7.36 (1H, m)	118.81
6’B	-	120.14	-	-
6’C	-	-	7.48 (1H, d, 8.59) and 7.49 (1H, d, 8.30)****	120.52

* There are two singlet peaks for this $H_{2'B}$, probably from two different configurations.
** There are two singlet peaks for this $H_{5'B}$, probably from two different configurations.
*** There are two sets of doublet for $H_{5'C}$, probably from two different configurations.
**** There are two sets of doublet for $H_{6'C}$, probably from two different configurations.