

Valonea Tannin: Tyrosinase Inhibition Activity, Structural Elucidation and Insights Into the Inhibition Mechanism

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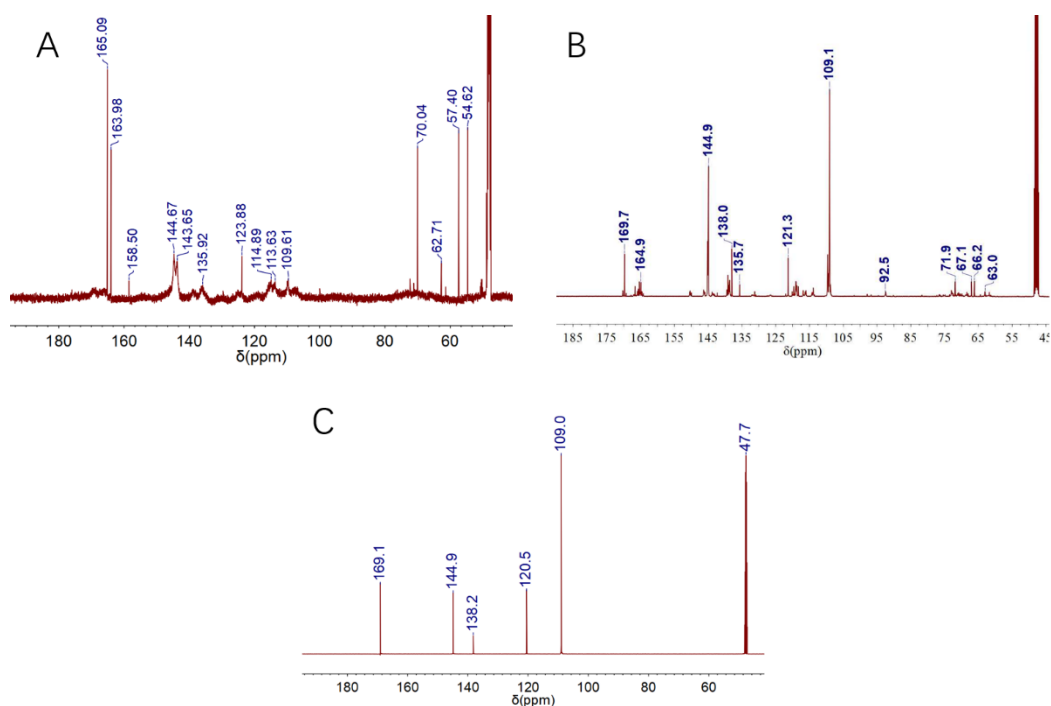


Figure S1. ¹³C NMR spectrum of valonia tannin (A), tannic acid (B) and gallic acid (C).

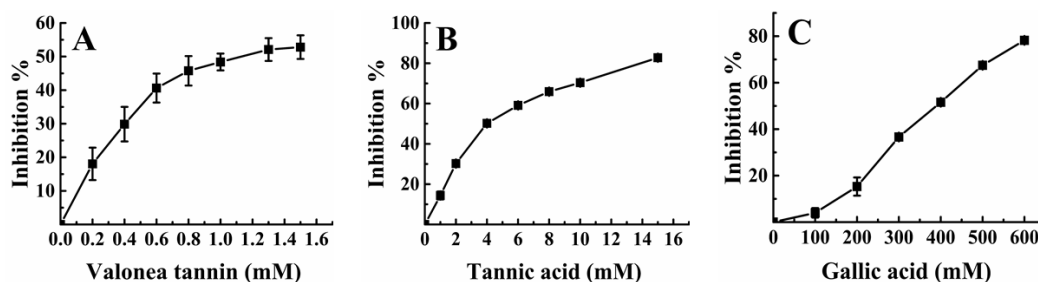


Figure S2. The tyrosinase inhibition activity was evaluated by conducting the catalysis reaction with different inhibitor concentrations. The inhibition rate-inhibitor concentration plots implied that, the inhibition rates (%) were positively related with the valonea tannin concentration (A), the tannic acid concentration (B) and the gallic acid (C) concentration respectively. These results implied that, the valonea tannin, tannic acid and gallic acid all presented tyrosinase inhibition ability, but their inhibitory concentration 50 (IC_{50}) were significantly different.

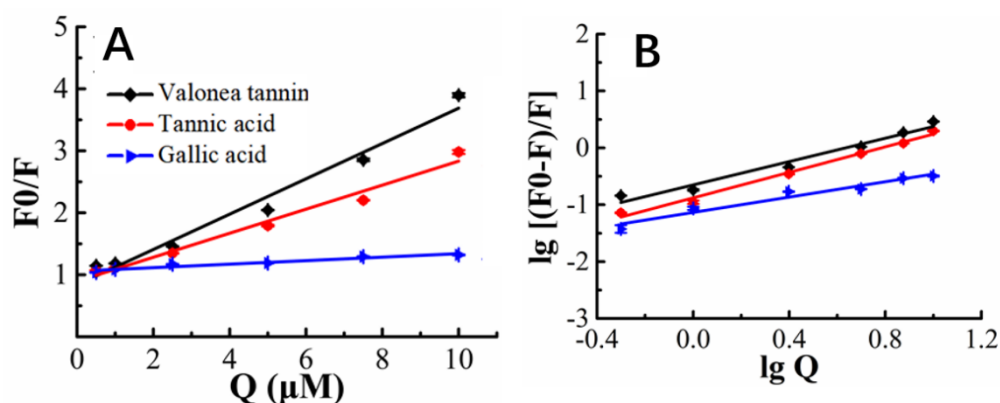


Figure S3. The Stern–Volmer plot (A, F_0/F against $[Q]$) and double log Stern–Volmer plot (B, $\log[(F_0 - F)/F]$ versus $\log[Q]$) obtained from calculating valonia tannin, tannic acid and gallic acid induced fluorescence quenching of the tyrosinase.

Table S4. The model fitting results from liner regression of the kinetic and fluorescence quenching analysis.

Tyrosinase concentration-reaction rate plots on Figure 4A, 4B and 4C								
Valonea tannin			Tannic acid			Gallic acid		
Line number	R^2	p	Line number	R^2	p	Line number	R^2	p
1	99.74	<0.00001	1	99.74	<0.00001	1	99.74	<0.00001
2	99.83	<0.00001	2	99.61	<0.00001	2	99.77	<0.00001
3	99.90	<0.00001	3	99.78	<0.00001	3	99.74	<0.00001
4	99.52	<0.00003	4	99.51	<0.00001	4	99.77	<0.00001
5	99.76	<0.00001	5	99.20	<0.00003	5	99.24	<0.00003
Lineweaver-Burk plots on Figure 4D, 4E and 4F								
Valonea tannin			Tannic acid			Gallic acid		
Line number	R^2	p	Line number	R^2	p	Line number	R^2	p
1	98.22	<0.0007	1	95.57	<0.003	1	92.00	<0.007
2	97.20	<0.002	2	97.15	<0.001	2	96.82	<0.002
3	99.27	<0.0001	3	99.08	<0.0003	3	95.94	<0.003
4	98.49	<0.0006	4	96.05	<0.002	4	92.94	<0.006
5	94.07	<0.004	5	99.12	<0.0002	5	94.89	<0.004
Enzyme-substrate complex (K_I) plots on Figure 4G, 4H and 4I								
Inhibitor	R^2	p	Inhibitor	R^2	p	Inhibitor	R^2	p
Valonea tannin	93.64	<0.005	Tannic acid	87.98	<0.01	Gallic acid	92.00	<0.006
Fluorescence quenching fitted by Stern-Volmer equation on Figure 5D								
Inhibitor	R^2	p	Inhibitor	R^2	p	Inhibitor	R^2	p
Valonea tannin	96.95	<0.0002	Tannic acid	98.80	<0.00004	Gallic acid	94.56	<0.0007
Stern-volmer equation fitted double logarithmic plot on Figure 5E								
Inhibitor	R^2	p	Inhibitor	R^2	p	Inhibitor	R^2	p
Valonea tannin	97.23	<0.0003	Tannic acid	98.80	<0.00004	Gallic acid	94.56	<0.0007

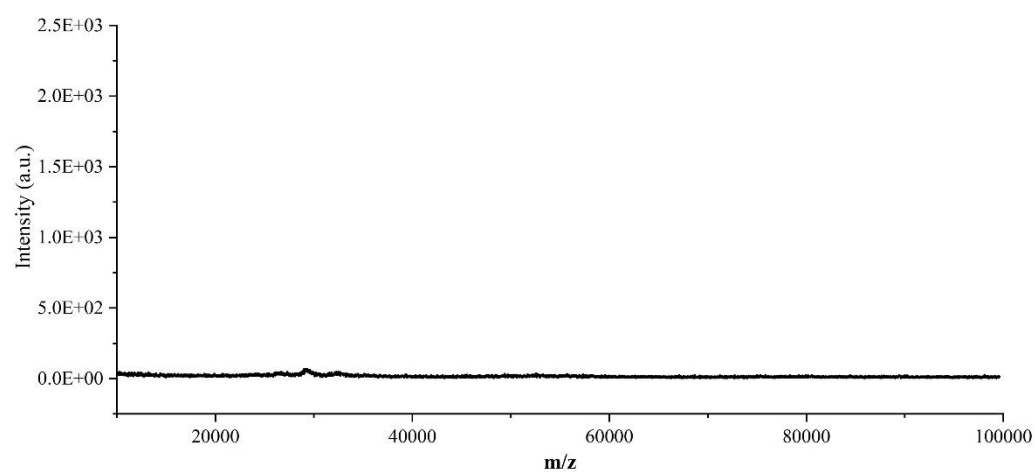


Figure S5. MALDI-TOF MS spectra of the purified tannin.

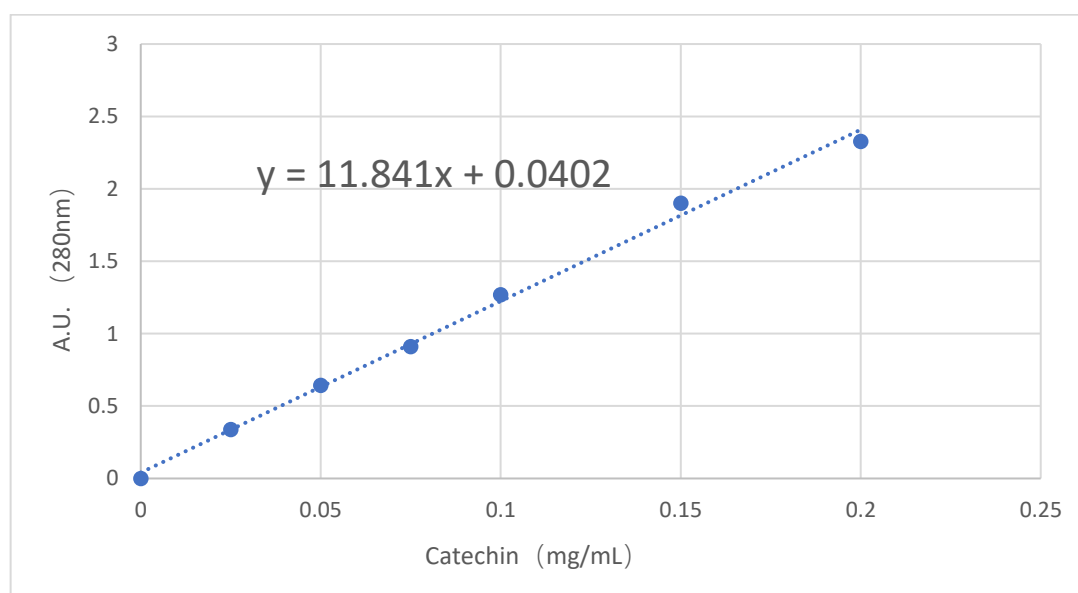


Figure S6. Standard curve of the methyl cellulose precipitation assay, where y is the absorbance at 280 nm and x is the tannin concentration (catechin equivalent).