

6'-O-Caffeoylarbutin from Quezui Tea: A Highly Effective and Safe Tyrosinase Inhibitor

Supplementary Materials

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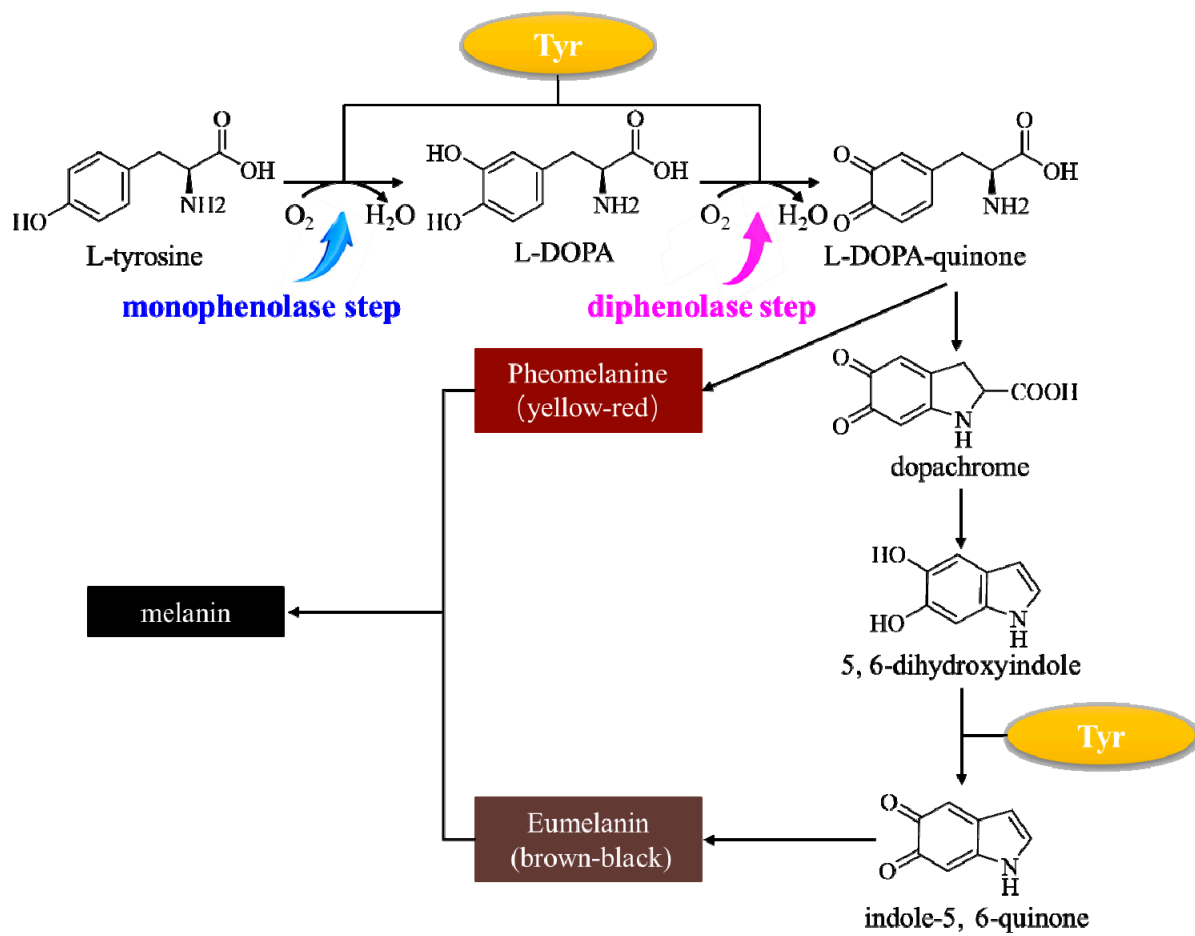


Figure S1. Melanin biosynthesis process

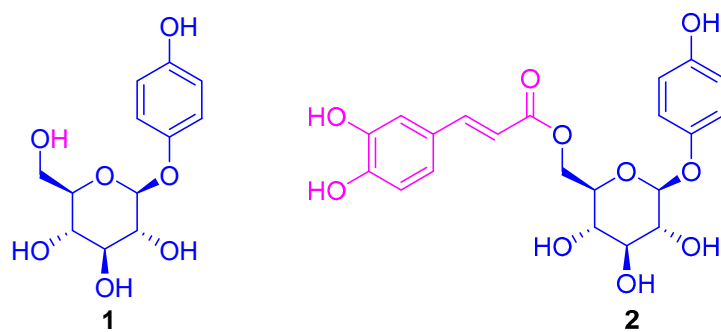


Figure S2. Chemical structures of *beta*-arbutin (1) and 6'-*O*-caffeoylarbutin (2)

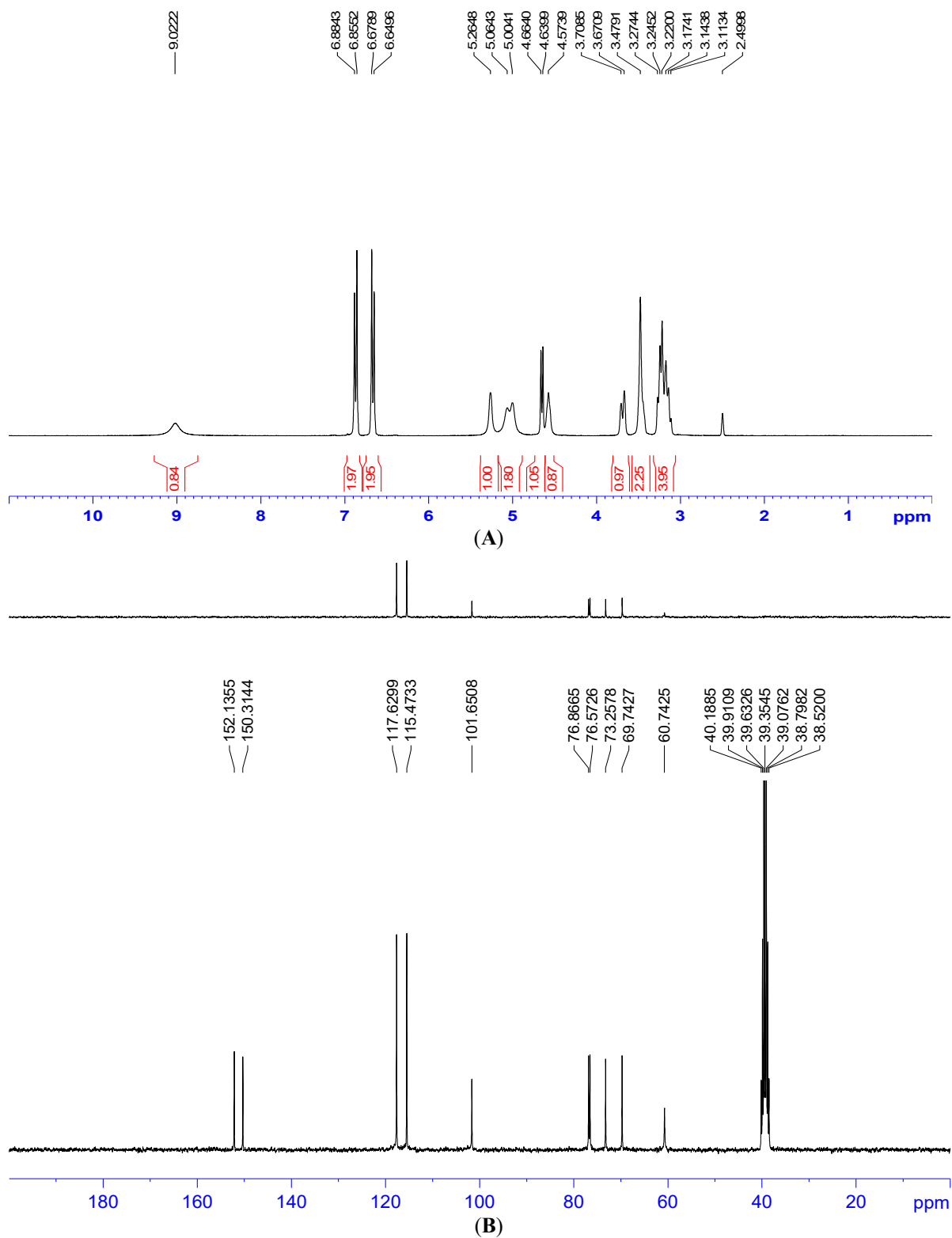
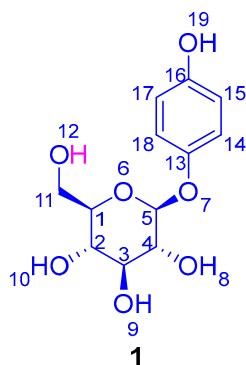


Figure S3. ^1H NMR (A) and ^{13}C NMR (B) spectra of *beta*-arbutin

^1H NMR and ^{13}C NMR assignments for *beta*-arbutin as follows:



beta-arbutin (**1**), white solid, ^1H NMR (300 MHz, DMSO) δ_{H} : 9.02 (H, s, 19-H), 6.86 (2H, d, $J = 8.6$ Hz, H-15, H-17), 6.66 (2H, d, $J = 8.6$ Hz, H-14, H-18), 5.26 (1H, s, H-5), 5.03 (2H, d, $J = 18.0$ Hz, H-8, H-10), 4.64 (1H, d, $J = 7.2$ Hz, H-9), 4.57 (1H, s, H-12), 3.69 (1H, d, $J = 11.7$ Hz, H-4), 3.48 (2H, s, H-2, H-3), 3.11-3.27 (3H, m, H-1, H-11); ^{13}C NMR (75 MHz, DMSO), δ_{C} : 152.1 (C-13), 150.3 (C-16), 117.6 (C-14, C-18), 115.5 (C-15, C-17), 101.7 (C-5), 76.9 (C-1), 76.6 (C-3), 73.3 (C-4), 69.7 (C-2), 60.7 (C-11).

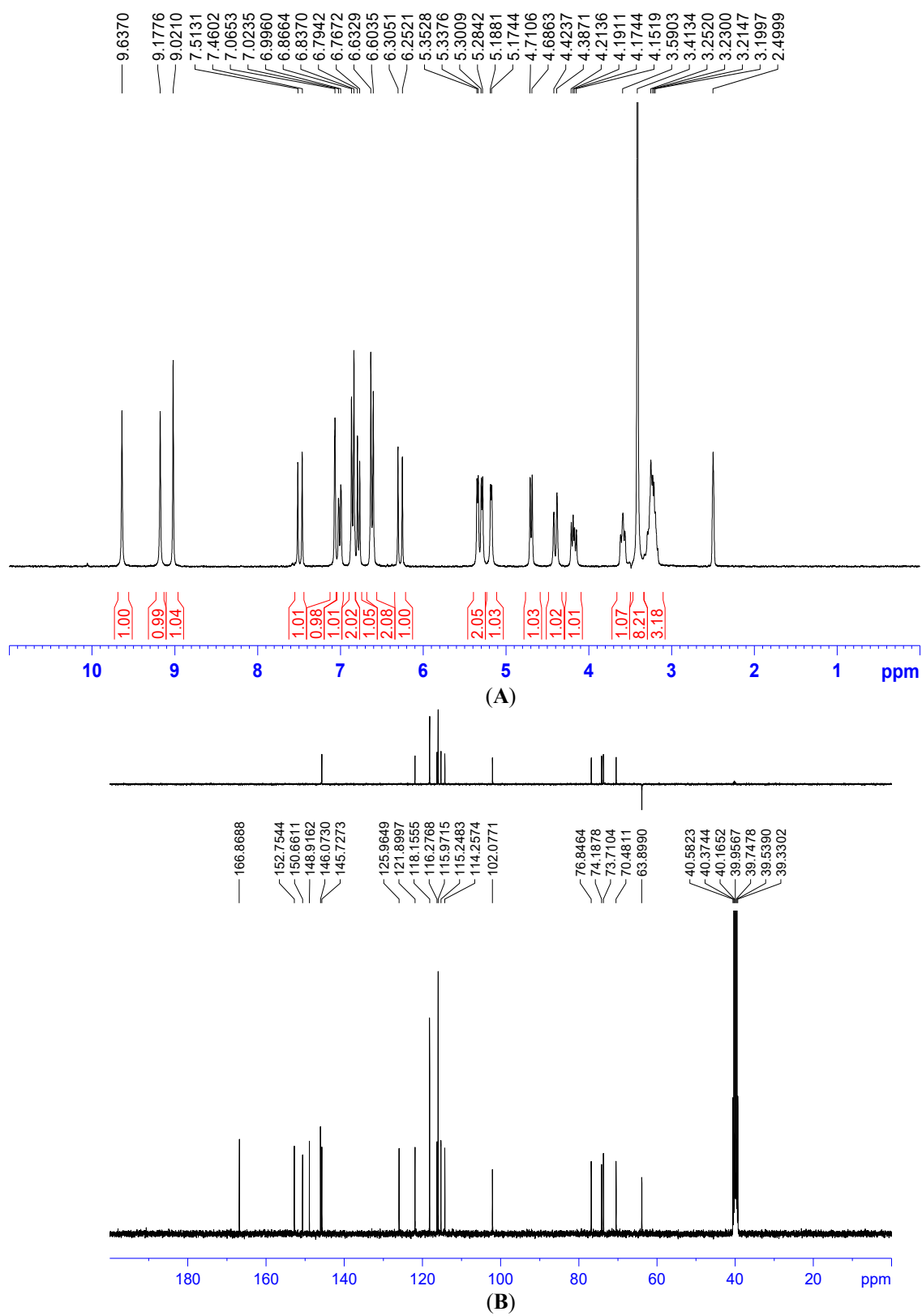
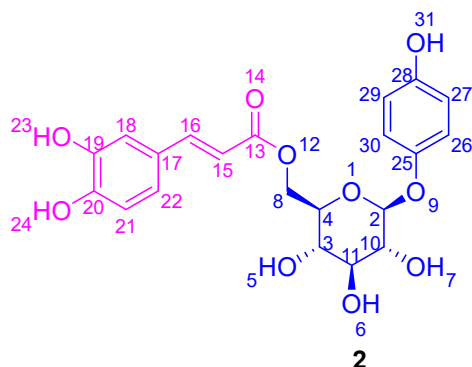
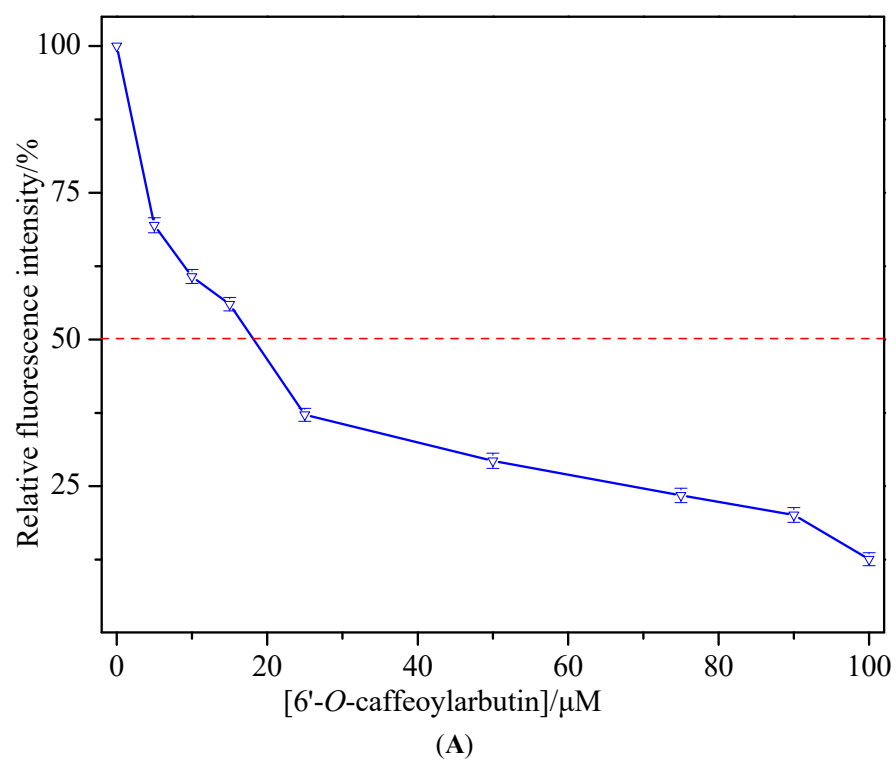


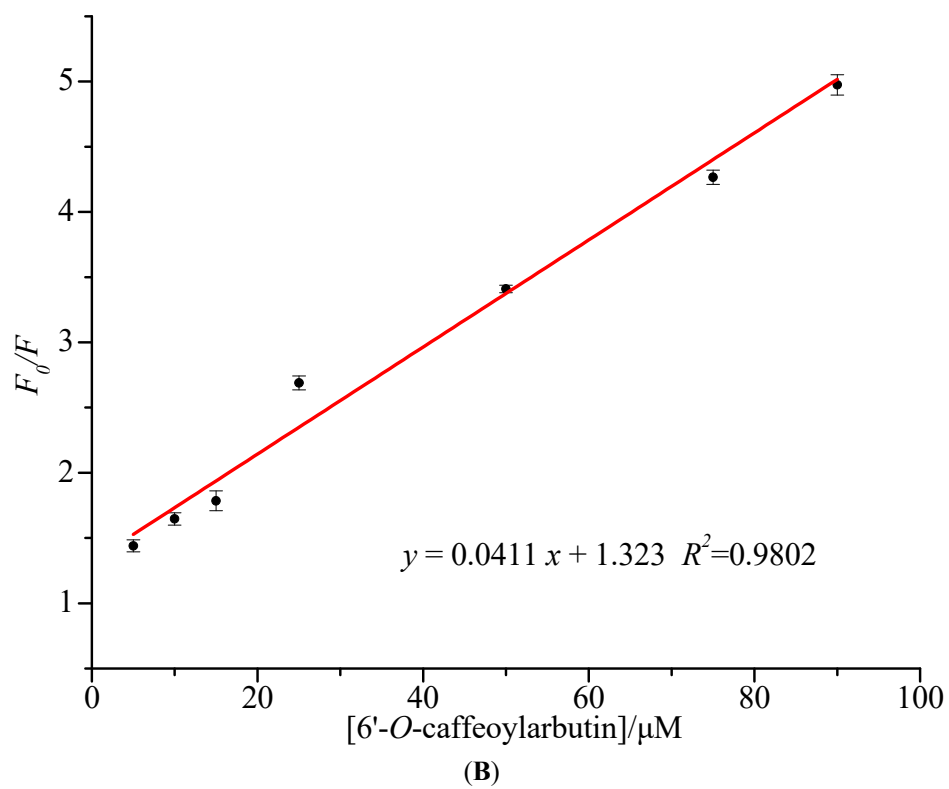
Figure S4. ^1H NMR (A) and ^{13}C NMR (B) for 6'-O-caffeoylarbutin

^1H NMR and ^{13}C NMR assignments for 6'-*O*-caffeoylarbutin as follows:



6'-*O*-caffeoylarbutin (**2**), colorless needles, ^1H NMR (300 MHz, DMSO) δ_{H} : 9.64 (1H, s, H-23), 9.18 (1H, s, H-24), 9.02 (1H, s, H-31), 7.48 (1H, d, $J = 15.9$, H-16), 7.05 (1H, s, H-18), 7.01 (1H, d, $J = 1.8$ Hz, H-21), 6.84 (1H, d, $J = 8.3$ Hz, H-22), 6.78 (2H, d, $J = 8.1$ Hz, H-27/29), 6.62 (2H, d, $J = 8.1$ Hz, H-26/30), 6.28 (1H, d, $J = 15.9$ Hz, H-15), 5.34 (2H, m, $J = 8.1$ Hz, H-8), 5.29 (1H, d, $J = 4.2$ Hz, H-2), 4.70 (1H, d, $J = 7.3$ Hz, H-10), 4.39 (1H, d, $J = 11.1$ Hz, H-11), 4.20 (1H, m, H-3), 3.59 (1H, t, H-4); ^{13}C NMR (75 MHz, DMSO), δ_{C} : 166.9 (C-13), 152.8 (C-25), 150.7 (C-28), 148.9 (C-20), 146.1 (C-19), 145.7 (C-16), 126.0 (C-17), 121.9 (C-22), 118.2 (C-26/30), 116.3 (C-15), 116.0 (C-27/29), 115.2 (C-18), 114.3 (C-21), 102.1 (C-2), 76.8 (C-4), 74.2 (C-11), 73.7 (C-10), 70.5 (C-3), 63.9 (C-8).





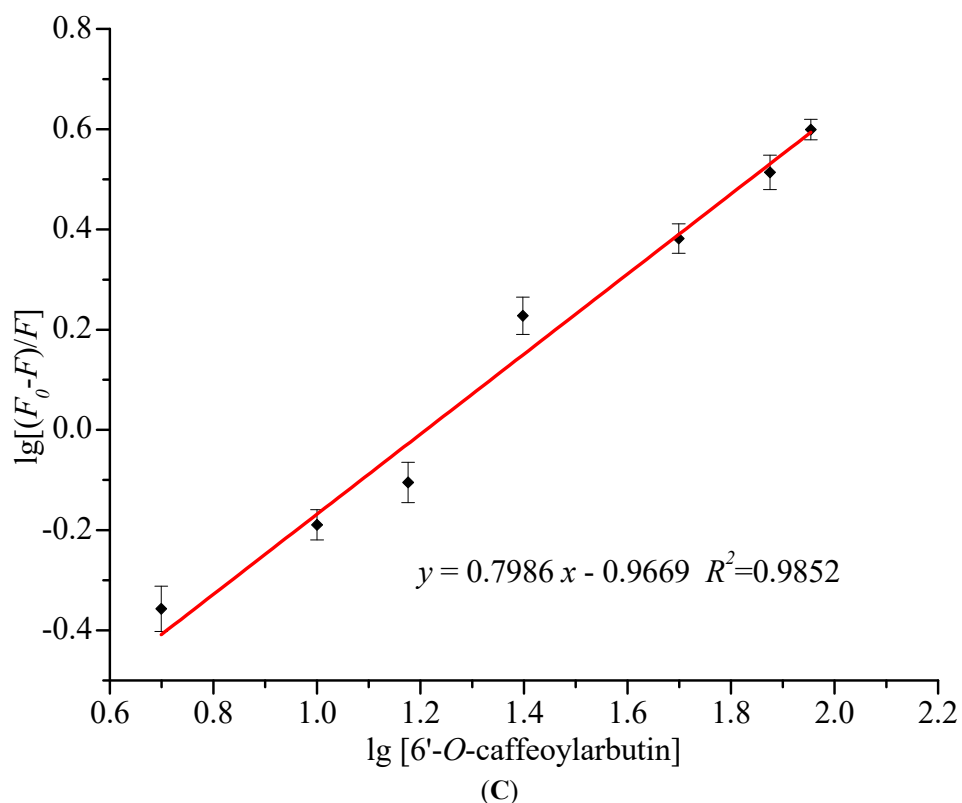


Figure S5. Fluorescence quenching effect of 6'-O-caffeoylarbutin on mTyr

(A). Relative fluorescence intensity of mTyr at different concentrations of 6'-O-caffeoylarbutin. (B). Stern-Volmer plot illustrating the fluorescence quenching of mTyr. C. Plot of $\lg [(F_0-F)/F]$ against $\lg [6'-O-caffeoylarbutin]$ for mTyr.

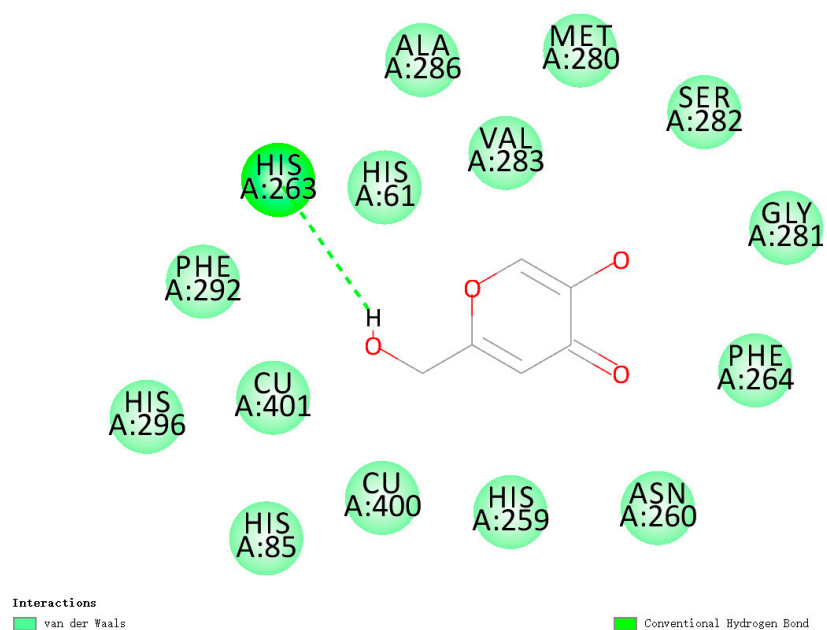
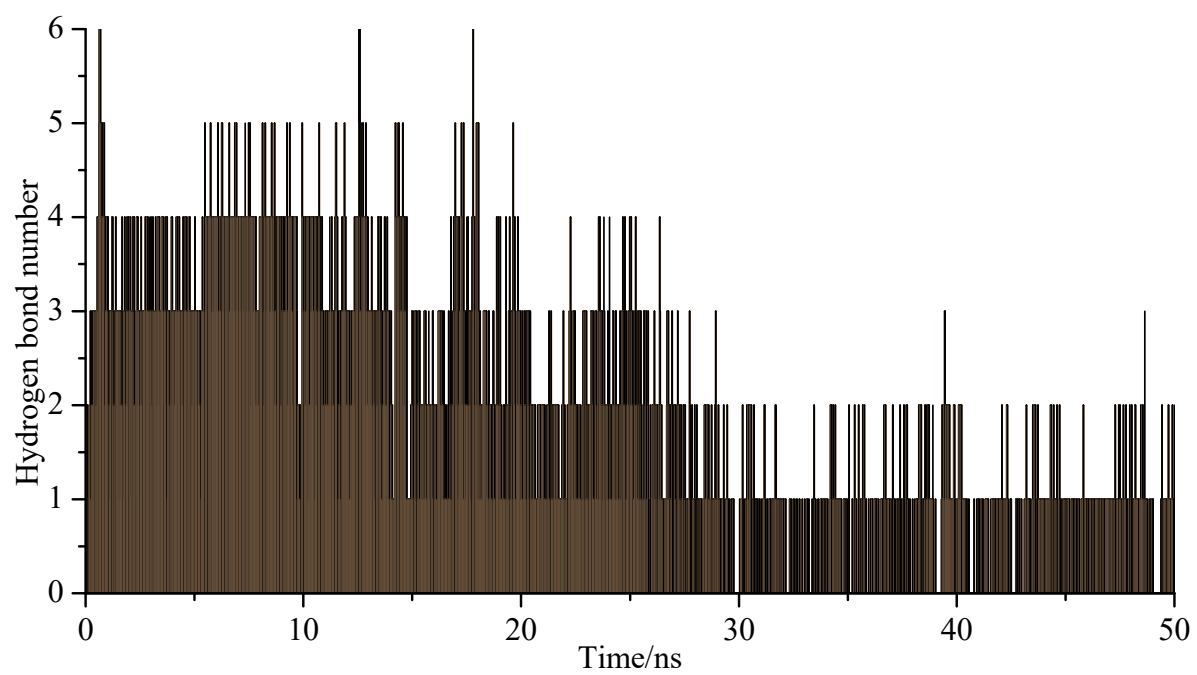
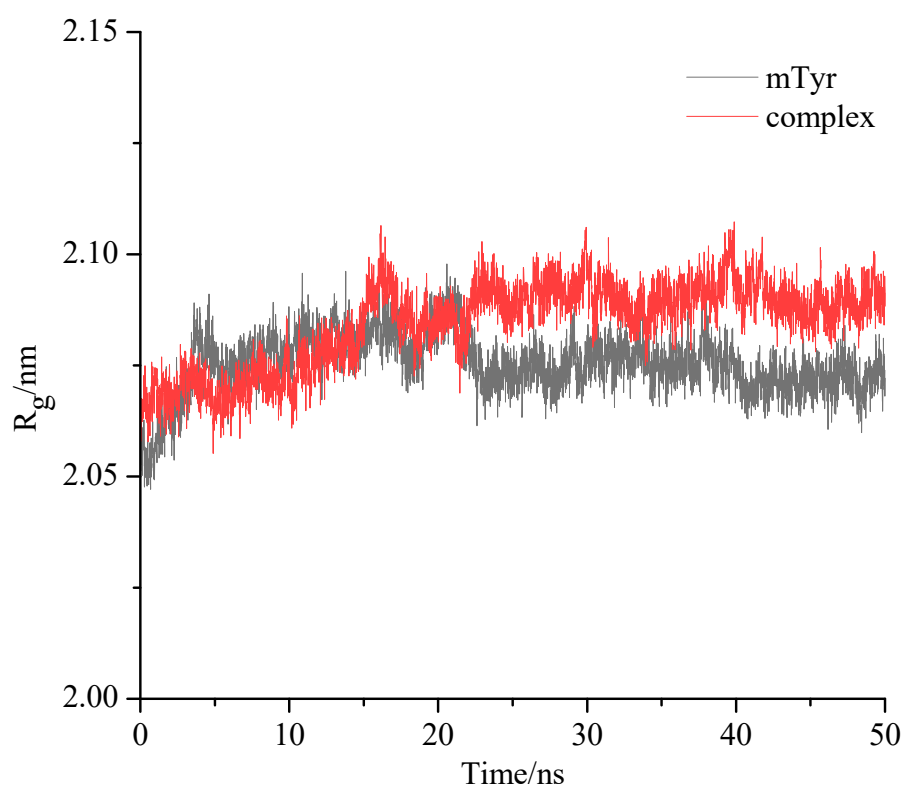


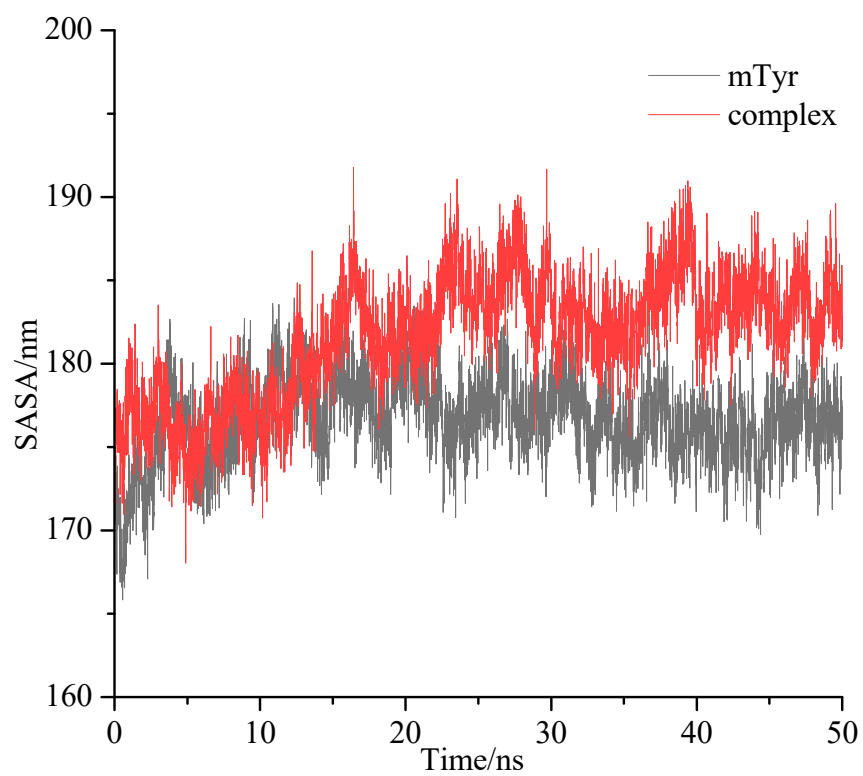
Figure S6. The docking of kojic acid with amino acid residue of pocket of mTyr.



(A)



(B)



(C)

Figure S7. Molecular dynamics (MD) simulation of 6'-*O*-caffeoylarbutin with mTyr
(A). The number of hydrogen bonds analysis. **(B).** Radius of gyration (Rg) change curve. **(C).** Solvent accessible surface area (SASA) change curve.

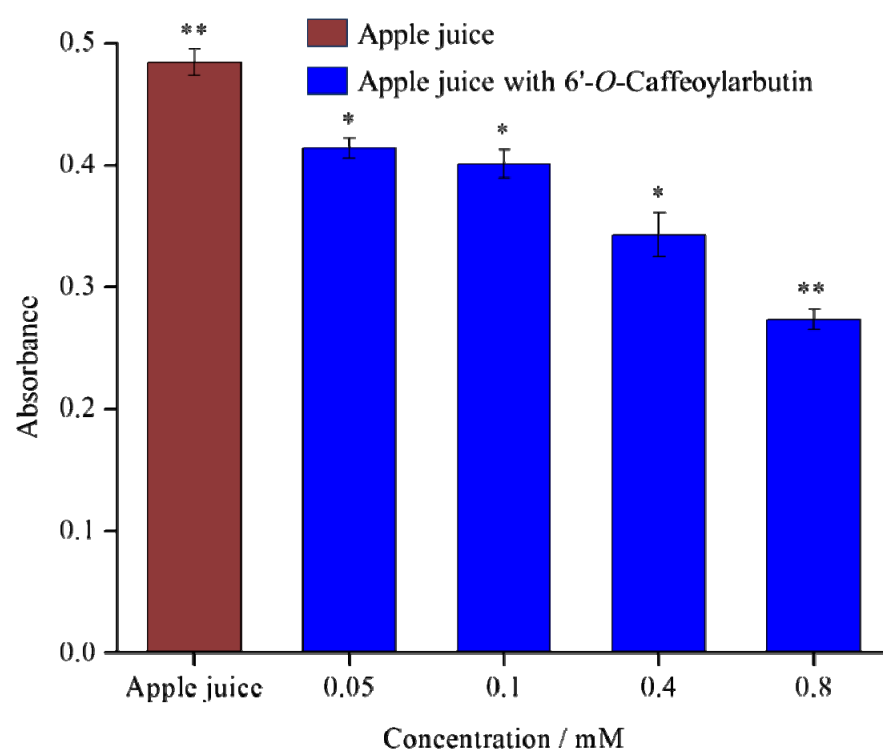


Figure S8. The browning inhibitory effect of 6'-O-caffeoylarbutin on apple juice

Table S1. mTyr inhibitory activities of 6'-*O*-caffeoylarbutin

Compound	IC ₅₀ ^a	
	Substrate [L-Tyrosine]	Substrate [L-DOPA]
6'- <i>O</i> -Caffeoylarbutin	1.1±0.1 µM	95.2±1.1µM
<i>beta</i> -Arbutin	8.4 mM	-

^aIC₅₀ values of all compounds are used as reference (Funayama et al., 1995 <https://doi.org/10.1271/bbb.59.143>; Liu et al., 2023 <https://doi.org/10.13386/j.issn1002-0306.2022070024>) and represent the concentration that caused 50% enzyme activity loss.

Table S2. Observation of hydrogen bonds in the 6'-*O*-caffeoylarbutin-2y9x protein complex obtained by molecular docking

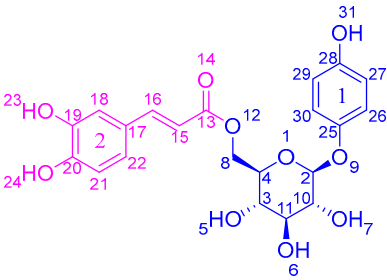
Amino acid species	6'- <i>O</i> -caffeoylarbutin	Atoms forming hydrogen bonds
Glu 322		hydrogen atom at position 23
His 85		hydrogen atom at position 7
Val 283		oxygen atom at position 7
Gly 281		oxygen atom at position 12
His 244		benzene ring 1

Table S3. The results acute test of 6'-*O*-caffeoylarbutin in mice (n=20)

Group	Number of mice	Final dose (mg·kg ⁻¹)	Death toll (♀/♂)	Death rate (%)
Vehicle	20	-	0/0	0
CA-1	20	28056	3/2	25
CA-2	20	23848	0/1	5
CA-3	20	20272	0/0	0

Table S4. Effect of 6'-*O*-caffeoylarbutin on body weight in mice

Group	Gender	Initial body weight	After the delivery				Body weight gain (g)
			1st day	3rd day	7th day	14th day	
Vehicle	Female	21.37±0.79	23.22±1.12	24.30±1.05	25.06±0.61	27.42±1.07	2.36±1.22
	Male	30.89±0.91	33.42±1.36	33.17±1.10	35.63±1.07	38.00±1.88	2.37±1.87
CA-1	Female	21.34±0.66	22.26±0.97	24.28±1.18	24.88±1.09	27.10±1.39	2.22±0.62
	Male	30.86±0.73	30.75±2.11**	33.62±2.35	35.25±2.53	37.90±3.13	2.65±1.01
CA-2	Female	21.20±0.60	21.58±1.21**	23.05±0.45**	23.59±0.50**	25.47±1.36	1.88±1.17
	Male	29.96±0.94	30.91±0.87**	33.07±1.24	33.88±1.71	37.26±2.19	3.38±1.23
CA-3	Female	21.27±0.58	22.21±0.87	23.36±0.66*	24.21±0.91*	26.62±1.54	2.41±1.51
	Male	30.32±0.88	30.89±1.57**	33.88±1.41	35.33±1.44	36.78±3.53	1.45±3.11

Statistical analysis: **/* had a significant difference compared with the Vehicle group ($p<0.05/0.01$).