

Supplementary File 1

1. Materials and Methods

The calculation method of icariin yield and epimedin C is as follows.

$$\text{Icariin Yield (\%)} = \frac{\Delta m \times Mr_1}{m_1 \times Mr_2} \times 100$$

$$\text{Epimedin C remained (\%)} = \frac{m_2}{m_1} \times 100$$

Δm : Mass difference of icariin before and after reaction.

m_1 : The quality of epimedin C before the reaction.

m_2 : The quality of epimedin C after the reaction.

Mr_1 : Relative molecular mass of epimedin C.

Mr_2 : Relative molecular mass of icariin.

2. Results

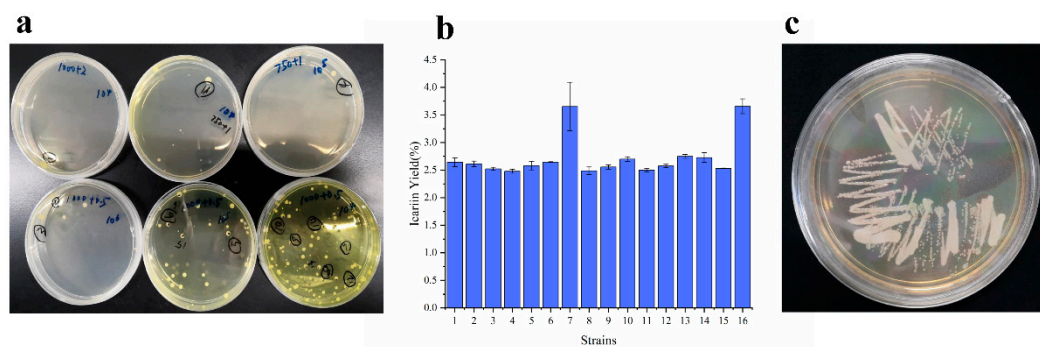
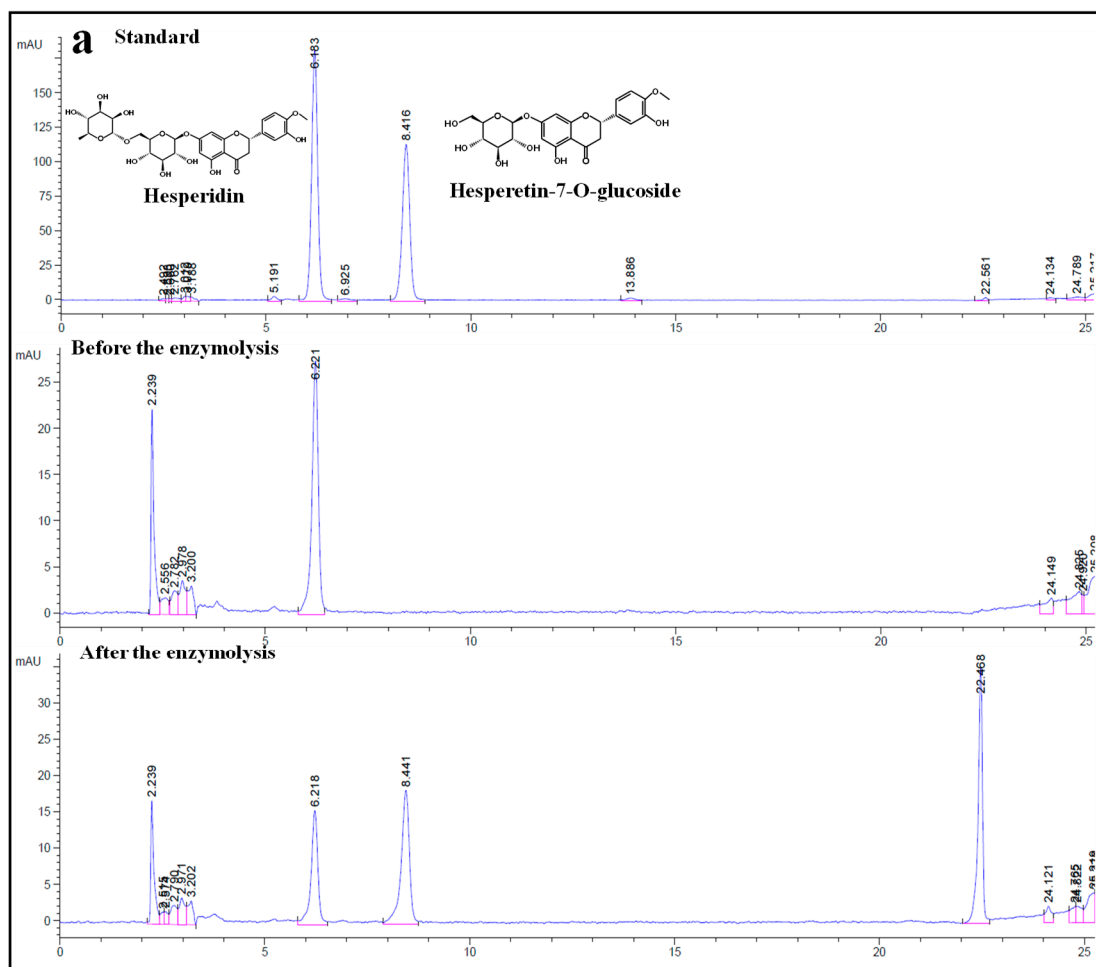
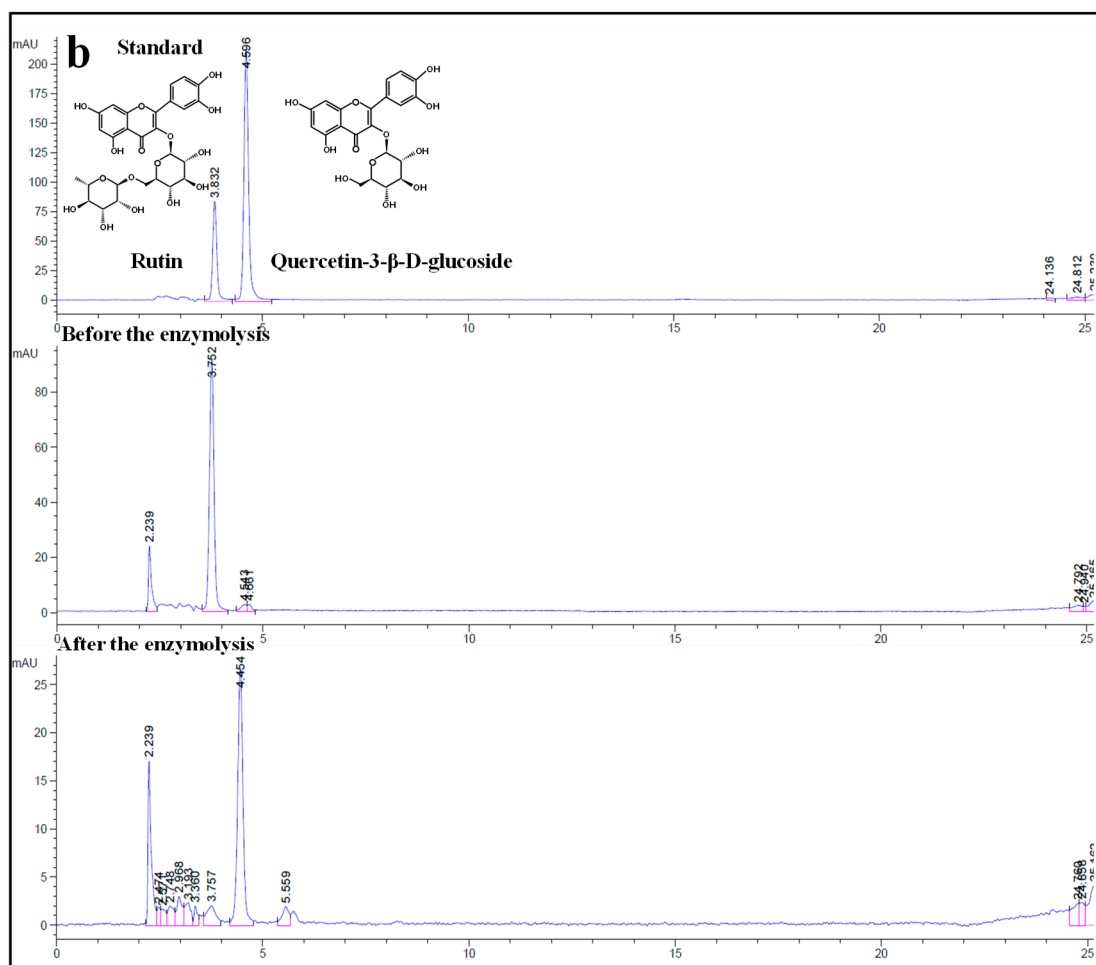
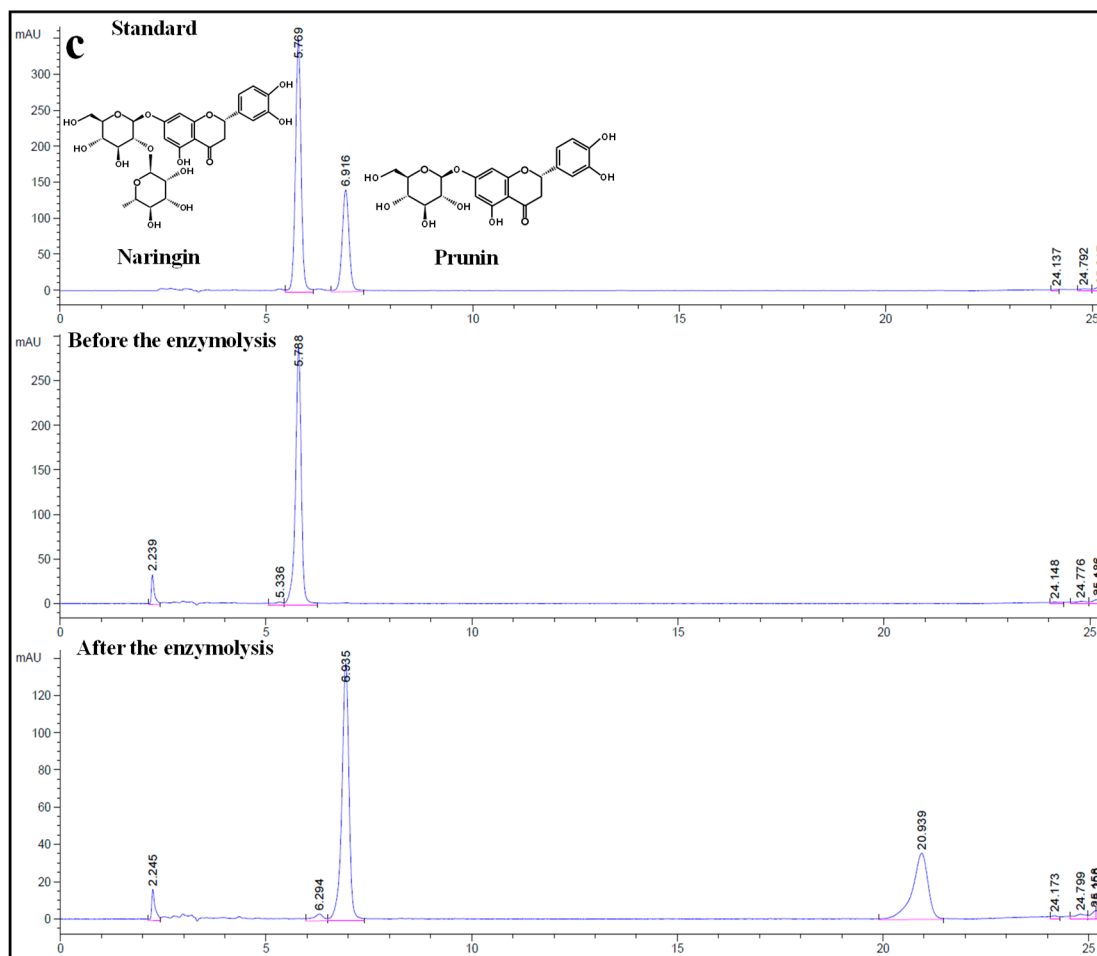
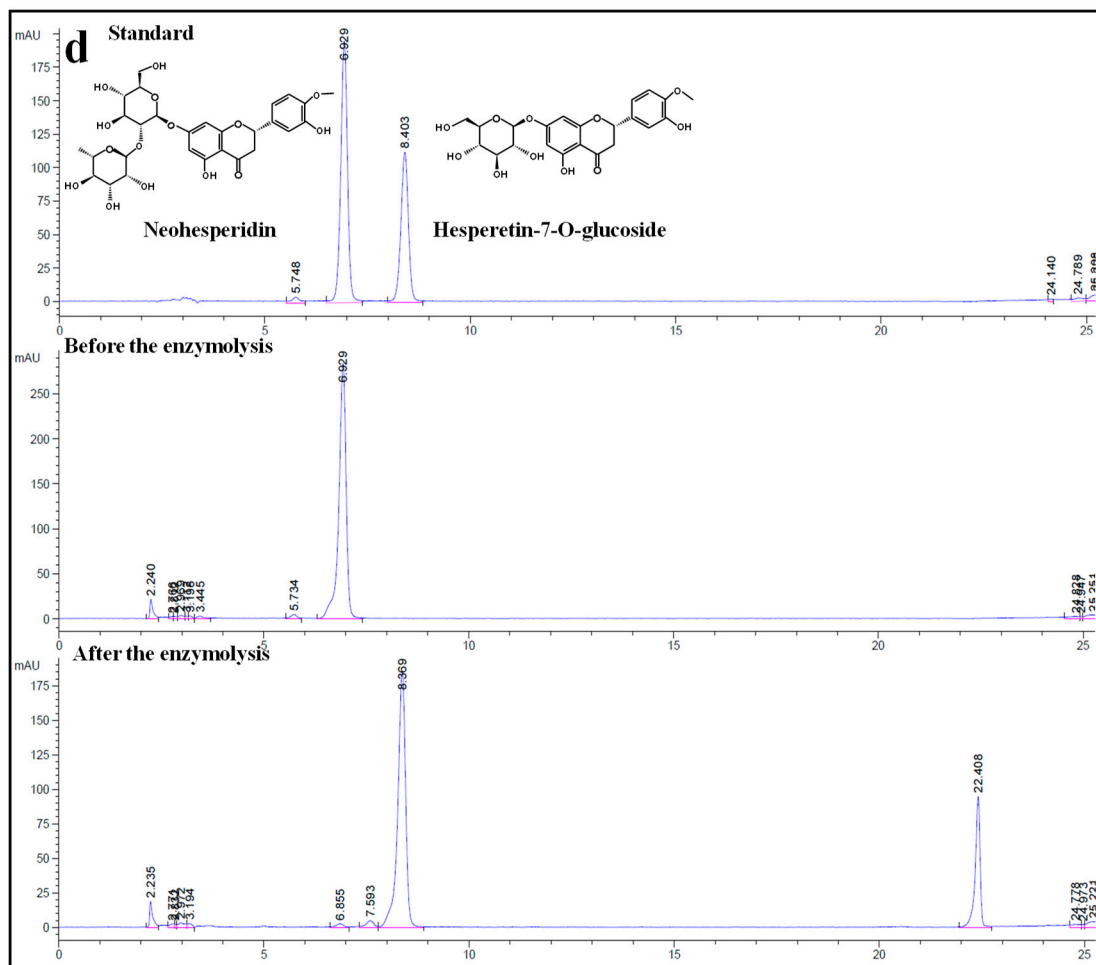


Figure S1 Strain screening results after mutagenesis for producing α -L-rhamnosidase transforming epimedin C. (a) Strains on LB medium including pNPR, (b) Icariin Yield, (c) The morphology of *P. laurentii* ZJU-L07 on YPD medium









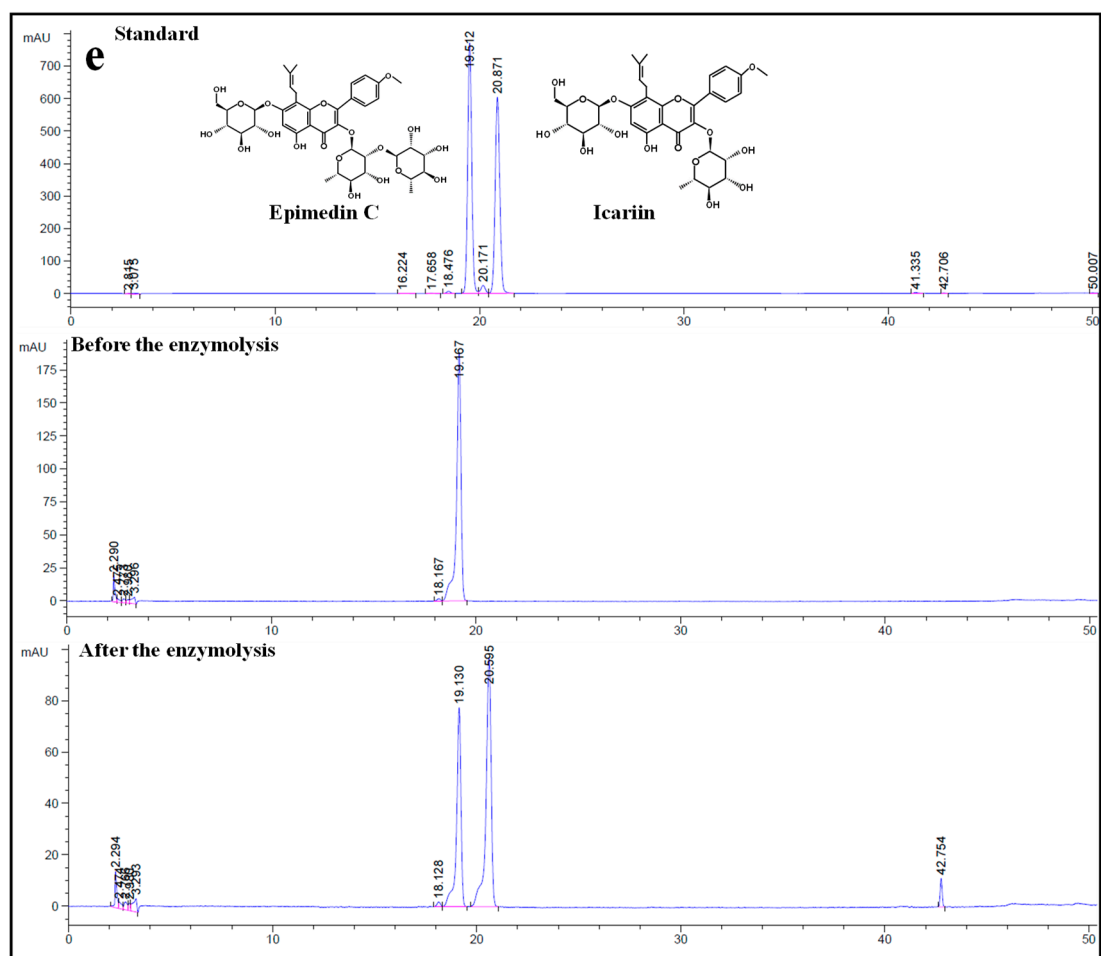


Figure S2 Chromatographs of the reaction mixtures before and after enzymatic hydrolysis of hesperidin(a), rutin(b), naringin(c), neohesperidin(d) and epimedin C(e).

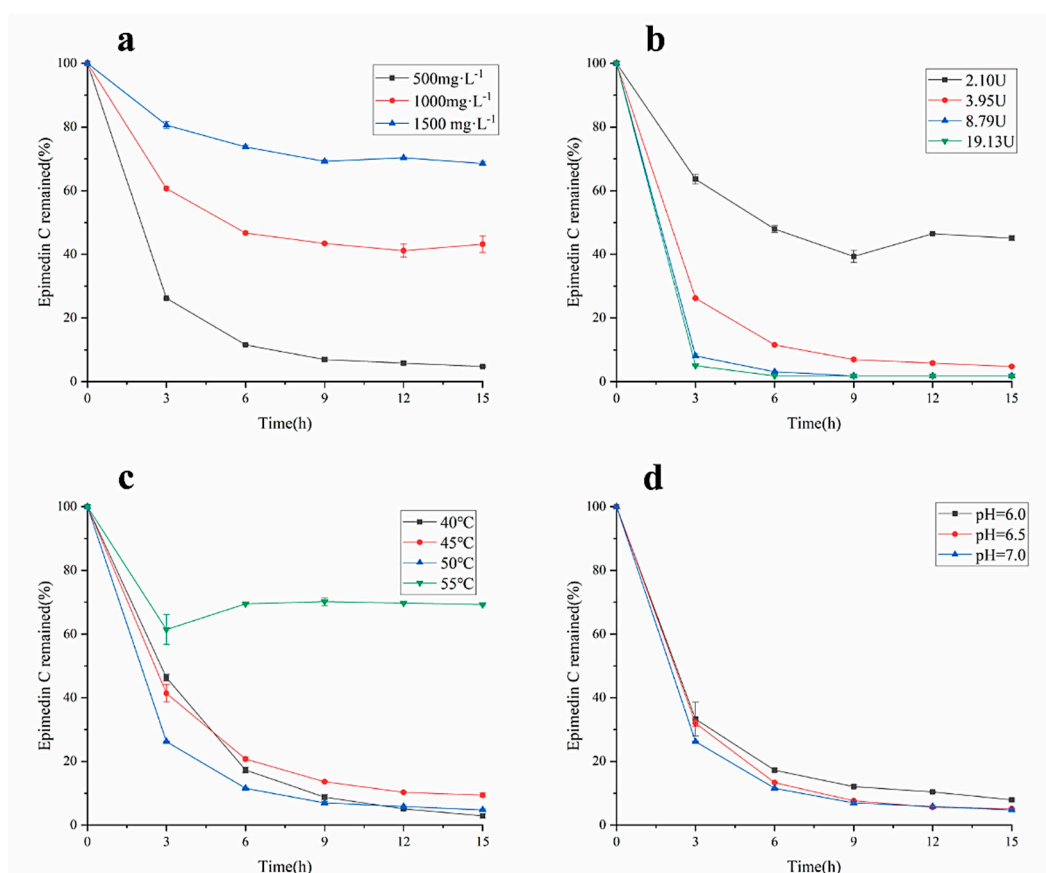


Figure S3 Enzymatic hydrolysis of EWE-II. (a) The amount of α -L-rhamnosidase on Epimedin C remained at 50 °C under pH 4.0 with 3.95 U of enzyme. (b) The amount of α -L-rhamnosidase on Epimedin C remained at 50 °C under pH 4.0 with 500 mg·L⁻¹ epimedin C. (c) Temperature affection with 3.95U of enzyme in the reaction system containing 500 mg·L⁻¹ epimedin C at pH 7.0. (d) pH affection with 3.95U of enzyme in the reaction system containing 500 mg·L⁻¹ epimedin C at 50 °C.

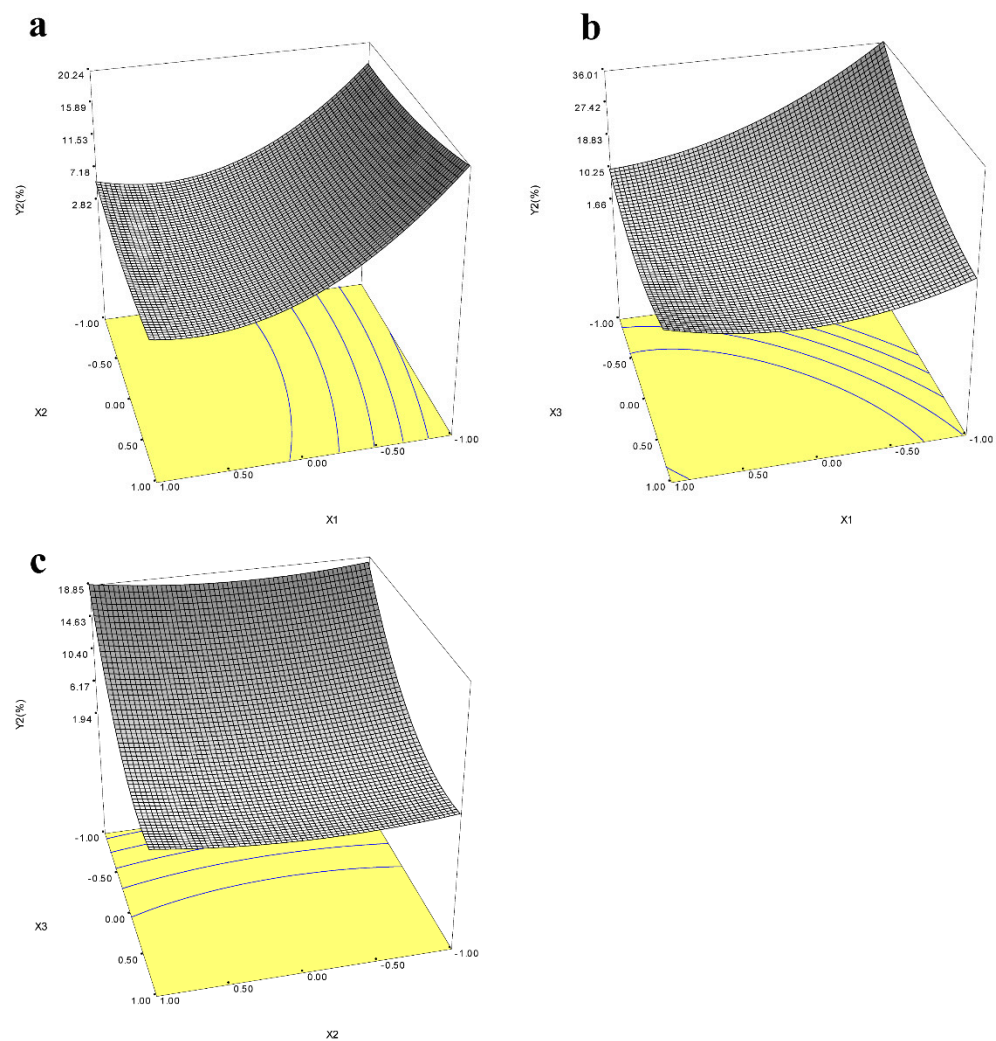


Figure S4 Response surface map of enzyme dosage (X_1), pH (X_2) and time (X_3) for Epimedin C remained (Y_2). Response surface maps of the interaction of enzyme activity (X_1) and pH (X_2) (a), enzyme activity (X_1) and hydrolysis time (X_3) (b), and pH (X_2) and hydrolysis time (X_3) (c) on Epimedin C remained (Y_2). The coordinate X represents the code value, not the actual value.

Table S1 ANOVA of the quadratic model for Epimedin C remained

Source	Sum of	Degree of	Mean	F- Value	p-value	Remarks
	Squares	freedom	Square		Probability > F	
Model	1256.579	9	139.620	22.529	0.0002	significant
X_1	392.305	1	392.3052	63.302	< 0.0001	
X_2	1.586	1	1.586	0.256	0.628	
X_3	458.441	1	458.441	73.973	< 0.0001	
X_1^2	135.017	1	135.017	21.786	0.0023	
X_2^2	5.220	1	5.220	0.842	0.389	
X_3^2	96.122	1	96.122	15.510	0.006	
$X_1 \times X_2$	4.195	1	4.195	0.677	0.438	
$X_1 \times X_3$	144.090	1	144.090	23.250	0.002	
$X_2 \times X_3$	0.009	1	0.009	0.0014	0.971	
Residual	43.382	7	6.197			
Lack of Fit	35.702	3	11.901	6.199	0.055	not significant
Pure Error	7.679	4	1.920			
Cor Total	1299.961	16				