

Supplementary Materials

1 Metabolomics profiling analysis of red clover

Table S1. Compound identification of red clover in positive and negative ion modes.

No	RT min	Ion Form	Ion Formula	MS ¹			MS ²	MS ³	Identification	Ste m	Le af	Flow er
				Measur ed m/z	Predicted m/z	Err ppm						
1	1.43	[M - H] ⁻	C ₆ H ₁₁ O ₇	195.050 5	195.0499	2.82			Gluconic acid	++	++	+++
2	4.46	[M - H] ⁻	C ₁₃ H ₁₁ O ₈	295.045 0	295.0448 4	0.67	179.03506(C₉H₇O₄); 133.01451(C ₄ H ₅ O ₅); 115.00400(C ₄ H ₃ O ₄);	[295-179]135.04524(C ₈ H ₇ O ₂);	Benzoylcitronensa ure	++	++ +	+++
3	5.42	[M + H] ⁺	C ₁₆ H ₁₉ O ₉	355.102 8	355.1024	1.21			chlorogenic acid	+	++ +	+++
4	6.60	[M + H] ⁺	C ₂₁ H ₂₁ O ₁₀	433.111 4	433.1129	-3.54	271.06067(C ₁₅ H ₁₁ O ₅);	[433-271]253.04956(C ₁₅ H ₉ O ₄); 243.06529(C ₁₄ H ₁₁ O ₄); 215.07042(C ₁₃ H ₁₁ O ₃); 153.01839(C₇H₅O₄); 149.02335(C ₈ H ₅ O ₃);	Genistein- glucoside	+++	++ +	+++
5	6.71	[M + H] ⁺	C ₂₁ H ₂₁ O ₉	417.117 0	417.1180	-2.34	239.07021(C ₁₅ H ₁₁ O ₃)		daidzin	+	++	+++
6	7.20	[M + H] ⁺	C ₂₂ H ₂₃ O ₁₀	447.127 7	447.1286	-1.91	269.08072(C ₁₆ H ₁₃ O ₄)		calycosin-7-O-β-D- glucoside	+++	++ +	+++

7	8.03	[M + H] ⁺	C ₂₅ H ₂₅ O ₁₃	533.127 1	533.1290	-3.54	285.07642(C ₁₆ H ₁₃ O ₅);	[533-285]270.05258(C ₁₅ H ₁₀ O ₅); 253.04982(C ₁₅ H ₉ O ₄); 225.05478(C ₁₄ H ₉ O ₃); 137.02333(C ₇ H ₅ O ₃);	Calycosin-7-O-β-D-glucoside 4''-O-malonate	+++	++ +	+++
8	8.95	[M + H] ⁺	C ₂₁ H ₂₁ O ₁₀	433.112 0	433.1129	-2.11	255.06516(C ₁₅ H ₁₁ O ₄)		genistin	++	++ +	+++
9	9.31	[M + H] ⁺	C ₂₄ H ₂₃ O ₁₃	519.111 6	519.1133	-3.25	433.11377(C ₂₁ H ₂₁ O ₁₀); 271.06073(C₁₅H₁₁O₅);	[519-271]253.04958(C ₁₅ H ₉ O ₄); 243.06526(C ₁₄ H ₁₁ O ₄); 215.07045(C₁₃H₁₁O₃); 153.01833(C ₇ H ₅ O ₄); 149.02333(C ₈ H ₅ O ₃);	Genistein-glucoside malonate	-	++ +	+++
10	9.64	[M + H] ⁺	C ₂₂ H ₂₁ O ₁₀	445.111 2	445.1129	-3.98	283.06073(C₁₆H₁₁O₅); 253.04929(C ₁₅ H ₉ O ₄); 225.05496(C ₁₄ H ₉ O ₃);		Pseudobaptigenin 7-O-β-D-glucoside	+++	++ +	+++
11	10.1 9	[M + H] ⁺	C ₂₂ H ₂₃ O ₉	431.132 3	431.1336	-3.18	269.08151(C₁₆H₁₃O₄); 254.05960(C ₁₅ H ₁₀ O ₄);	[431-269] 254.05771(C₁₅H₁₀O₄); 213.09125(C ₁₄ H ₁₃ O ₂); 137.02341(C ₇ H ₅ O ₃); 136.01564(C ₇ H ₄ O ₃);	Ononin	+	++ +	+++
12	10.5	[M + H] ⁺	C ₂₅ H ₂₅ O ₁₄	549.122 5	549.1239	-2.52	463.12491(C ₂₂ H ₂₃ O ₁₁); 301.07153(C₁₆H₁₃O₆);	[549-301]286.04773(C₁₅H₁₀O₆); 269.04495(C ₁₅ H ₉ O ₅); 245.08113(C ₁₄ H ₁₃ O ₄); 241.04996(C ₁₄ H ₉ O ₄); 153.01846(C ₇ H ₅ O ₄); 149.02351(C ₈ H ₅ O ₃);	Trihydroxy-methoxyflavone-glucoside-malonate	-	++ +	+++
13	11.0 2	[M - H] ⁻	C ₇ H ₅ O ₃	137.024 03	137.0233	5.18			Salicylic acid	+++	++ +	+++

14	11.4 7	[M + H] ⁺	C ₁₅ H ₁₁ O ₄	255.064	255.0652	-4.65	237.05475(C ₁₅ H ₉ O ₃);	199.07558(C ₁₃ H ₁₁ O ₂);	Daidzein	+	++	+++	
				0			209.05980(C ₁₄ H ₉ O ₂);				+		
15	12.1 6	[M + H] ⁺	C ₂₅ H ₂₃ O ₁₃	531.111	531.1133	-3.29	227.07045(C ₁₄ H ₁₁ O ₃);	283.06085(C ₁₆ H ₁₁ O ₅);	[531-283]253.04999(C ₁₅ H ₉ O ₄);	Pseudobaptigenin	+++	++	++
				6			225.05502(C ₁₄ H ₉ O ₃);		226.06264(C ₁₄ H ₁₀ O ₃);	197.05977(C ₁₃ H ₉ O ₂);		7-O-β-glucoside	
16	12.2 8	[M + H] ⁺	C ₂₂ H ₂₁ O ₁₁	461.106	461.1078	-2.36	209.05980(C ₁₄ H ₉ O ₂);	299.05402(C ₁₆ H ₁₁ O ₆);	[461-299]269.04468(C ₁₅ H ₉ O ₅);	Deirisin	-	++	+++
				8			241.04973(C ₁₄ H ₉ O ₄);		225.05496(C ₁₄ H ₉ O ₃);	181.01332(C ₈ H ₅ O ₅);		-4'-O-β-D-glucoside	
17	12.6 5	[M + H] ⁺	C ₂₅ H ₂₅ O ₁₂	517.132	517.1340	-3.93	137.02345(C ₇ H ₅ O ₃);	269.08173(C ₁₆ H ₁₃ O ₄);	[517-269]254.05789(C ₁₅ H ₁₀ O ₄);	Formononetin 7-O-	+	++	+++
				0			237.05495(C ₁₅ H ₉ O ₃);		226.06264(C ₁₄ H ₁₀ O ₃);	213.09138(C ₁₄ H ₁₃ O ₂);		β-D-glucoside -O-	
18	13.7 6	[M + H] ⁺	C ₂₂ H ₂₃ O ₁₀	447.127	447.1286	-3.27	137.02356(C ₇ H ₅ O ₃);	285.07651(C ₁₆ H ₁₃ O ₅);	[447-285]270.05261(C ₁₅ H ₁₀ O ₅);	Biochanin A-β-D-	+++	++	+++
				1			229.08620(C ₁₄ H ₁₃ O ₃);		152.01059(C ₇ H ₄ O ₄);	glucoside		+	
19	14.3	[M + H] ⁺	C ₂₂ H ₂₃ O ₁₀	447.127	447.1286	-3.56	229.08606(C ₁₄ H ₁₃ O ₃);	285.07626(C ₁₆ H ₁₃ O ₅);	[447-285]257.08096(C ₁₅ H ₁₃ O ₄);	Prunetin	+++	++	+++
				0			167.03397(C ₈ H ₇ O ₄);		175.03906(C ₁₀ H ₇ O ₃);	-4'-O-β-D-glucoside		+	

20	14.6	[M + H] ⁺	C ₂₅ H ₂₃ O ₁₄	547.106 3	547.1082	-3.49	299.05591(C₁₆H₁₁O₆);	151.03908(C ₈ H ₇ O ₃); 123.04417(C₇H₇O₂); [547-299]269.04462(C₁₅H₉O₅); 243.06537(C ₁₄ H ₁₁ O ₄); 241.04973(C ₁₄ H ₉ O ₄); 225.05486(C ₁₄ H ₉ O ₃); 181.01329(C ₈ H ₅ O ₅);	Deirisin -4'-O-β-D- glucoside -O-malonate	+	++ +	+++
21	15.8	[M + H] ⁺	C ₁₅ H ₁₁ O ₅	271.059 2	271.0601	-3.32	253.04976(C ₁₅ H ₉ O ₄); 243.06548(C ₁₄ H ₁₁ O ₄); 215.07062(C ₁₃ H ₁₁ O ₃); 153.01849(C₇H₅O₄);	Genistein	-	++ +	+++	
22	15.8	[M + H] ⁺	C ₂₅ H ₂₅ O ₁₃	533.126 5	533.1290	-4.70	447.13031(C ₂₂ H ₂₃ O ₁₀); 285.07681(C₁₆H₁₃O₅);	Biochanin A-β-D- glucoside-O- malonate	+++	++ +	+++	
23	16.3	[M + H] ⁺	C ₂₅ H ₂₅ O ₁₃	533.127 44	533.1290	-2.87	285.0749(C₁₆H₁₃O₅); 286.04752(C₁₅H₁₀O₆); 269.04486(C ₁₅ H ₉ O ₅); 241.05003(C ₁₄ H ₉ O ₄); 153.01851(C ₇ H ₅ O ₄);	Biochanin A-β-D- glucoside-O- malonate isomer	+++	++ +	+++	
24	16.9	[M + H] ⁺	C ₁₆ H ₁₃ O ₆	301.069 2	301.0707	-4.83	269.04486(C ₁₅ H ₉ O ₅); 241.05003(C ₁₄ H ₉ O ₄); 153.01851(C ₇ H ₅ O ₄);	Pratensein	-	++ +	+++	

25	18.7	[M + H] ⁺	C ₁₆ H ₁₁ O ₅	283.058	283.0601	-4.66	253.04979(C₁₅H₉O₄);	Pseudobaptigenin	-	++	+++
				8			225.05496(C ₁₄ H ₉ O ₃);			+	
							197.05974(C ₁₃ H ₉ O ₂);				
							183.04405(C ₁₂ H ₇ O ₂);				
26	19.2	[M + H] ⁺	C ₁₆ H ₁₃ O ₄	269.080	269.0808	-3.25	254.05817(C₁₅H₁₀O₄);	Formononetin	+	++	+++
				0			241.08643(C ₁₅ H ₁₃ O ₃);			+	
							237.05533(C ₁₅ H ₉ O ₃);				
							213.09163(C ₁₄ H ₁₃ O ₂);				
27	21.9	[M + H] ⁺	C ₁₆ H ₁₁ O ₆	299.053	299.0550	-4.56	269.04492(C₁₅H₉O₅);	Irilone	+	++	+++
				7			243.06557(C ₁₄ H ₁₁ O ₄);			+	
							241.04990(C ₁₄ H ₉ O ₄);				
							225.05492(C ₁₄ H ₉ O ₃);				
28	24.7	[M + H] ⁺	C ₁₆ H ₁₃ O ₅	285.074	285.0758	-3.23	270.05292(C₁₅H₁₀O₅);	Biochanin A	-	++	+++
				8			257.08127(C ₁₅ H ₁₃ O ₄);			+	
							253.05009(C ₁₅ H ₉ O ₄);				
							229.08647(C ₁₄ H ₁₃ O ₃);				
		153.01862(C ₇ H ₅ O ₄);									
		152.01076(C ₇ H ₄ O ₄);									
		149.02357(C ₈ H ₅ O ₃);									
		123.04431(C ₇ H ₇ O ₂);									

Bold characters: the base peaks in MSⁿ spectra; -: means not detected. +++: the response value of the sample is 80–100% of the maximum; ++: the response value of the sample is 40–80% of the maximum; +: the response value of the sample is within 40% of the maximum.

2. Blood-absorbed components analysis for the validation of the quality control markers

Generally, traditional herbal medicine is administered orally, so the components that are absorbed into blood take effect. Therefore, the nine potential quality control markers screened by network pharmacology were further confirmed in the plasma by UPLC-MS.

The nine potential quality control markers screened by network pharmacology were extracted by exact mass search in the plasma, using the high accuracy of m/z determined by UPLC-MS. All the nine compounds were successfully found and conformed as shown in Table S1. The identification was confirmed by comparing their UPLC retention times and MS data with the authentic commercial standards.

Table 2. Blood-absorbed components analysis for the validation of the quality control markers.

No.	RT min	Ion Form	molecular Formula	MS ¹			Identification
				Measured m/z	Predicted m/z	Err ppm	
1	10.06	[M + H] ⁺	C ₂₂ H ₂₂ O ₉	431.1329	431.1336	-1.69	ononin
2	11.22	[M + H] ⁺	C ₁₅ H ₁₀ O ₄	255.0638	255.0652	-5.51	daidzein
3	5.22	[M + H] ⁺	C ₁₆ H ₁₈ O ₉	355.1011	355.1024	-3.56	chlorogenic acid
4	6.53	[M + H] ⁺	C ₂₁ H ₂₀ O ₉	417.1162	417.1180	-4.38	daidzin
5	7.01	[M + H] ⁺	C ₂₂ H ₂₂ O ₁₀	447.1266	447.1286	-4.41	calycosin-7-O-β-D-glucoside
6	15.51	[M + H] ⁺	C ₁₅ H ₁₀ O ₅	271.0585	271.0601	-5.79	Genistein
7	19.00	[M + H] ⁺	C ₁₆ H ₁₂ O ₄	269.0793	269.0808	-5.78	Formononetin
8	8.75	[M + H] ⁺	C ₂₁ H ₂₀ O ₁₀	433.1109	433.1129	-4.51	genistin
9	24.47	[M + H] ⁺	C ₁₆ H ₁₂ O ₅	285.0738	285.0758	-6.77	Biochanin A

3 The results of correlation analysis for the contents of nine compounds and the total contents

Table S3. The results of correlation analysis

		Chlorogenic Acid	Daidzin	Calycosin-7-O- β -D-glucoside	Genistin	Ononin	Daidzein	Genistein	Formononetin	Biochanin A	Total Content
Chlorogenic acid	Pearson correlation	1	0.017	0.735**	0.344	0.497	-0.485	0.122	0.025	0.227	0.486
	<i>P</i> value (bilateral)		0.958	0.006	0.273	0.100	0.110	0.706	0.938	0.478	0.109
	<i>N</i>	12	12	12	12	12	12	12	12	12	12
Daidzin	Pearson correlation	0.017	1	0.191	0.588*	0.379	0.407	0.414	-0.019	0.122	0.439
	<i>p</i> value (bilateral)	0.958		0.495	0.021	0.164	0.132	0.125	0.947	0.665	0.102
	<i>N</i>	12	15	15	15	15	15	15	15	15	15
calycosin-7-O- β -D-glucoside	Pearson correlation	0.735**	0.191	1	0.358	0.534*	-0.242	-0.094	0.082	0.119	0.532*
	<i>p</i> value (bilateral)	0.006	0.495		0.190	0.040	0.384	0.740	0.772	0.673	0.041
	<i>N</i>	12	15	15	15	15	15	15	15	15	15
Genistin	Pearson correlation	0.344	0.588*	0.358	1	0.895**	-0.353	0.464	-0.031	0.446	0.793**
	<i>p</i> value (bilateral)	0.273	0.021	0.190		0.000	0.197	0.081	0.912	0.096	.000
	<i>N</i>	12	15	15	15	15	15	15	15	15	15
Ononin	Pearson correlation	0.497	0.379	0.534*	0.895**	1	-0.494	0.314	0.129	0.368	0.849**

	<i>p</i> value (bilateral)	0.100	0.164	0.040	0.000		0.061	0.254	0.647	0.178	0.000
	<i>N</i>	12	15	15	15	15	15	15	15	15	15
Daidzein	Pearson correlation	-0.485	0.407	-0.242	-0.353	-0.494	1	0.267	0.250	-0.024	-0.186
	<i>p</i> value (bilateral)	0.110	0.132	0.384	0.197	0.061		0.337	0.369	0.933	0.507
	<i>N</i>	12	15	15	15	15	15	15	15	15	15
Genistein	Pearson correlation	0.122	0.414	-0.094	0.464	0.314	0.267	1	0.540*	0.762**	0.637*
	<i>p</i> value (bilateral)	0.706	0.125	0.740	0.081	0.254	0.337		0.038	0.001	0.011
	<i>N</i>	12	15	15	15	15	15	15	15	15	15
Formononetin	Pearson correlation	0.025	-0.019	0.082	-0.031	0.129	0.250	0.540*	1	0.634*	0.534*
	<i>p</i> value (bilateral)	0.938	0.947	0.772	0.912	0.647	0.369	0.038		0.011	0.041
	<i>N</i>	12	15	15	15	15	15	15	15	15	15
Biochanin A	Pearson correlation	0.227	0.122	0.119	0.446	0.368	-0.024	0.762**	0.634*	1	0.735**
	<i>p</i> value (bilateral)	0.478	0.665	0.673	0.096	0.178	0.933	0.001	0.011		0.002
	<i>N</i>	12	15	15	15	15	15	15	15	15	15
Total content	Pearson correlation	0.486	0.439	0.532*	0.793**	0.849**	-0.186	0.637*	0.534*	0.735**	1
	<i>p</i> value (bilateral)	0.109	0.102	0.041	0.000	0.000	0.507	0.011	0.041	0.002	
	<i>N</i>	12	15	15	15	15	15	15	15	15	15

****.** $p < 0.01$; *****. $p < 0.05$
