

**Network pharmacology-based study on the lipid-lowering effect of *Bidens bipinnata* L.
extract with experimental validation in a hyperlipidemia rat model**

Revista Brasileira de Farmacognosia

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Supplementary Material

HPLC-Q-TOF/MS Method for the Analysis of *Bidens bipinnata* L. (BBL) Extract

Analysis conditions: Poroshell 120 EC-C18 column (3 mm × 150 mm, 2.7 μm); mobile phase: acetonitrile (A)–0.2% formic acid (B), gradient elution (0–1 min, 5%–15% A; 1–7 min, 15%–25% A; 7–11 min, 25%–40% A; 11–14 min, 40%–50% A; 14–15 min, 50%–100% A; 15–20 min, 100% A; 20–21 min, 100%–5% A); flow rate 0.5 mL/min; column temperature 35 °C; injection volume 5 μL. G6530A Q-TOF/MS mass spectrometer; AJS ESI source; ESI+ and ESI– modes; drying gas temperature 320 °C; drying gas flow rate 8 L/min; nebulizer pressure 40 psi (1 psi = 6.895 kPa); sheath gas temperature 300 °C; sheath gas flow rate 12 L/min; fragmentor voltage 175 V; capillary voltage 3.5 kV; mass range m/z 100–1700 in positive and negative ion modes.

HPLC-Q-TOF/MS Result for the Analysis of BBL Extract

Fig. S1 Total ion chromatograms under positive (A, B, C) and negative (D, E, F) ion modes for blank serum (A, D), serum containing BBL decoction (B, E), and BBL decoction itself (C, F). Peaks: 1, gallic acid; 2, protocatechuic acid; 3, chlorogenic acid; 4, 3,4-di-O-caffeoylquinic acid; 5, 3,5-di-O-caffeoylquinic acid; 6, rutin; 7, cynaroside; 8, hyperoside; 9, bipinnatapolyacetyloside; 10, luteolin; 11, quercetin.

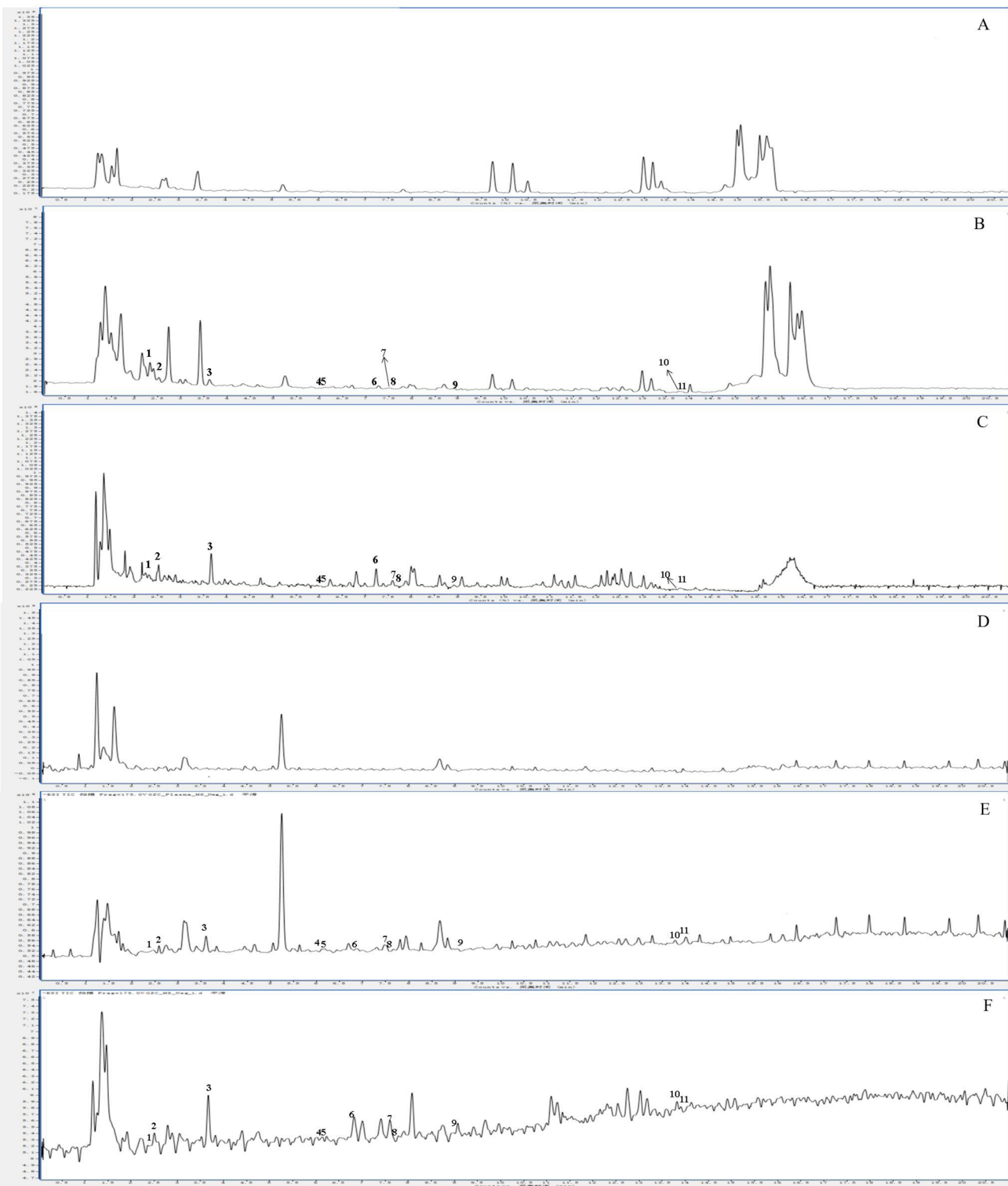


Table S1 Identification of chemical compounds from BBL in rat serum by HPLC-Q-TOF/MS

No.	RT(min)	Molecular Formula	[M-H] ⁻		[M+H] ⁺		MS/MS		Proposed Compound
			Theoretical m/z	Experimental m/z	Theoretical m/z	Experimental m/z	ESI ⁽⁻⁾	ESI ⁽⁺⁾	
1	2.412	C ₇ H ₆ O ₅	169.0142	169.0146	171.0293	171.0291	151.0033 [M-H-H ₂ O] ⁻ 125.0236 [M-H-CO ₂] ⁻	127.0395 [M+H-CO ₂] ⁺	Gallic acid ^a
2	2.503	C ₇ H ₆ O ₄	153.0193	153.0196	155.0344	155.0341	108.0211 [M-H-CHO ₂] ⁻ , 109.0294 [M-H-CO ₂] ⁻	111.0446 [M+H-CO ₂] ⁺	Protocatechuic acid ^a
3	3.657	C ₁₆ H ₁₈ O ₉	353.0878	353.0874	355.1029	355.1026	179.0344 [M-H-C ₇ H ₁₀ O ₅] ⁻ , 135.0446 [M-H-C ₇ H ₁₀ O ₅ -CO ₂] ⁻	181.0501 [M+H-C ₇ H ₁₀ O ₅] ⁺	Chlorogenic acid ^a
4	6.156	C ₂₅ H ₂₄ O ₁₂	515.1190	515.1188	517.1346	517.1344	353.0876 [M-H-C ₉ H ₈ O ₄] ⁻ 191.0558 [M-H-C ₁₈ H ₁₆ O ₈] ⁻	355.1031 [M+H-C ₉ H ₇ O ₃] ⁺	3,4-di-O-caffeoylquinic acid
5	6.223	C ₂₅ H ₂₄ O ₁₂	515.1190	515.1193	517.1346	517.1348	179.0343 [M-H-C ₁₆ H ₁₈ O ₉] ⁻ 353.0875 [M-H-C ₉ H ₈ O ₄] ⁻ 191.0554 [M-H-C ₁₈ H ₁₆ O ₈] ⁻	355.1027 [M+H-C ₉ H ₇ O ₃] ⁺ 163.0395 [M+H-C ₁₆ H ₁₇ O ₉] ⁺	3,5-di-O-caffeoylquinic acid
6	6.816	C ₂₇ H ₃₀ O ₁₆	609.1456	609.1457	611.1612	611.1606	179.0341 [M-H-C ₁₆ H ₁₈ O ₉] ⁻ 463.0878 [M-H-C ₆ H ₁₀ O ₄] ⁻ 301.0344 [M-H-C ₁₃ H ₂₂ O ₉] ⁻	303.0507 [M+H-C ₁₂ H ₂₀ O ₉] ⁺	Rutin ^a
7	7.655	C ₂₁ H ₂₀ O ₁₂	463.0877	463.0876	465.1033	465.1031	193.0134 [M-H-C ₁₃ H ₂₂ O ₉ -B] ⁻ 107.0135 ^{0,4} A ⁻ 301.0349 [M-H-C ₆ H ₁₂ O ₆] ⁻	303.0503 [M+H-C ₆ H ₁₂ O ₆] ⁺	Hyperoside ^a
8	7.853	C ₂₁ H ₂₀ O ₁₁	447.0927	447.0925	449.1084	449.1082	257.0452 [M-H-C ₆ H ₁₂ O ₆ -CO ₂] ⁻ 107.0133 ^{0,4} A ⁻	287.0554 [M+H-C ₆ H ₁₂ O ₆] ⁺	Cynaroside ^a
9	8.982	C ₁₉ H ₂₂ O ₈	377.1236	377.1241	379.1393	379.1396	285.0397 [M-H-C ₆ H ₁₂ O ₆] ⁻ 267.0291 [M-H-H ₂ O] ⁻ 217.0498 [M-H-C ₃ O ₂] ⁻	401.1215 [M+Na] ⁺ 217.0871 [M+H-C ₆ H ₁₂ O ₆] ⁺ 181.0712 [M+H-C ₁₃ H ₁₂ O ₃] ⁺	Bipinnatapolycetyloside
10	13.934	C ₁₅ H ₁₀ O ₆	285.0399	285.0401	287.0556	287.0553	267.0291 [M-H-H ₂ O] ⁻ 241.0503 [M-H-CO ₂] ⁻ 217.0498 [M-H-C ₃ O ₂] ⁻	269.0452 [M+H-H ₂ O] ⁺	Luteolin ^a
11	14.127	C ₁₅ H ₁₀ O ₇	301.0348	301.0351	303.0505	303.0503	257.0454 [M-H-CO ₂] ⁻ 193.0133 [M-H-B] ⁻	275.0553 [M+H-CO] ⁺ 259.0608 [M+H-CO ₂] ⁺	Quercetin ^a

Note: ^a indicates comparison with reference standards.

Table S2 Information on primer sequences

Gene name	Primer F (5' -3')	Primer R (5' -3')
CYP1A2	GCCCTGAAGAGTTTCTCCATAG	AGCTTCTGGAAGTTGCTGATTA
CYP3A4	CACTTTCCTTTGTCCTGCATTC	TAGGTGGGAGGTGCCTTAT
HMGCR	AAGAGTCGCTGTGTTCATCTC	CCTGCTTGTAAGTCTGCTCTAAC
RXRA	GCTCCTCAGGCAAACACTAT	CAGGTGTAGGTCAGGTCTTTG
RXRB	CCTTCCCAGTCATCAGTTCTTC	GAGCGACACTGTGGAGTTAAT
APOA2	CTGTAGCCTAGAAGGAGCTTTG	TCCATCAAATCCTTGCCATAGT
GAPDH	GCAAGGATACTGAGAGCAAGAG	GGATGGAATTGTGAGGGAGATG