1.Preparation method

Weigh 1 g of the freeze-dried powder of mulberry prepared in the laboratory, add 5 mL of 50% (V/V) methanol-water solution, and place the supernatant in a high-speed centrifuge at 13,000 r/min for 10 minutes to obtain the mulberry extract. The same operation steps are used to obtain the blank control sample.

2.Instrument parameter

2.1 Chromatographic condition

The sample obtained by the above method is filtered using an SPFE filter (waters); then 4 μL is injected into the HPLC, and qualitative and quantitative UHPLC-MS analysis of the extract is performed using the UltiMate 3000 UHPLC (Thermo Fisher Scientific, USA) system. This system is equipped with a 2.1 mm × 100 mm, 1.8 μm, C-18 column, with a column temperature of 45 °C. A linear gradient solvent system of acetonitrile (solvent A) and 0.1% (V/V) formic acid in aqueous solution (solvent B) is used as follows: 5% solution A, 0-1 min; 5%-95% solution A, 1-30 min; 95% solution A, 30-32 min; 95%-5% solution A, 32-32.10 min; 5% solution A, 32.10-35 min. The monitoring wavelength range is 210-800 nm.

2.2Mass spectrum condition

The mass spectrometer used in this study is the LTQ ORBITRAP VELOS PRO (Thermo Fisher Scientific, USA), operating under chromatographic conditions as previously described. In positive ion mode, the HESI source operates with an ion source temperature of 350 °C, a capillary temperature of 320 °C, a sheath gas flow rate of 35 units, an auxiliary gas flow rate of 10 units, a spray voltage of 4 kV, a capillary voltage of 35 V, and a tube lens voltage of 110 V. Samples undergo an initial full scan with a resolution set at 30,000 and a scan range of 50-900 m/z. Secondary scans utilize dynamic data-dependent scanning (DDS), selecting the top three most abundant peaks from the previous stage for collision-induced dissociation (CID) fragment scanning, detected with a dynode in the ion trap. In negative ion mode, the ion source temperature is 300 °Cthe ion source temperature is 300℃, the capillary temperature is 320℃, the sheath gas flow rate is 35 units, the auxiliary gas flow rate is 10 units, the spray voltage is 3.6kV, and the capillary voltage is 35V, with the tube lens voltage at 110V, while the other parameters remain the same as in positive ion mode.

No.	Name	Adduct	m/z (Apex)	m/z (Delta(ppm))	Measured Area	Left RT
1	Epicatechin	M+H	212.05505	-1.39723	6950665.377	1.335738
2	Pyroglutamic acid	M+H	130.05	-2.0275	4710981.778	1.381
3	2-formyl-1h-pyrrorole-1-butyric acid	M+H	126.055	-1.85913	4584789.053	1.3242
4	2α, 3β-dihydroxynorepinephrine	M+H	182.081	-1.30372	4542174.946	1.4803
5	Protocatechuic acid	M-H	153.0199	3.708	2967111.19	2.8399
6	arabinose	M-H	109.03	3.945	2346913.537	2.8399
7	2- (5-hydroxymethyl-2-formylpyrrole-1-yl) propionic acid	M+H	180.065	-0.92525	1852241.853	1.699
8	morin	M-H	301.0358	1.386	1552914.336	10.504
9	Quercetin	M-H	301.0358	1.386	1552914.336	10.504
10	Ethyl pyroglutamic acid	M+H	158.081	-1.11555	1370277.429	2.5534
11	2β, 3β-dihydroxynorepinephrine	M+H	144.102	-1.03493	1024135.191	1.347
12	2- (5-hydroxymethyl-2-methylpyrrole-1-yl) propionic acid lactone	M+H	144.102	-1.03493	1024135.191	1.347
13	glucose	M-H	179.057	3.39	937009.4046	0.914
14	Astragaloside IV	M-H	179.057	3.39	937009.4046	0.914
15	P-hydroxybenzoic acid	M+H	139.039	-1.09063	893790.232	1.5635
16	Ethyl caffeic acid	M+H	209.081	-1.01663	666091.2502	6.8238
17	2-[2-formyl-5 (-methoxy methyl) -1H-pyrrolo-1-yl]-3- (4-hydroxyphenyl) propionic acid methyl ester	M+H	198.076	-1.23093	516201.1351	1.1293
18	Methyl ester of 3-caffeoyl quinic acid	M+H	369.1174	-1.64955	437430.7241	7.4713
19	Quercetin 3-O-galactoside	M+H	369.1174	-1.64955	437430.7241	7.4713
20	scopoletin	M+H	193.049	-0.68397	361649.5549	9.9453
21	Aesculin	M+H	179.034	-1.87631	289288.0591	1.7654
22	Catechol	M+H	179.034	-1.87631	289288.0591	1.7654
23	Dihydroquercetin	M-H	303.052	2.301	267274.8556	7.7148
24	Caffeyl quinic acid	M+H	355.102	-2.07395	249959.0449	6.6723
25	Chlorogenic acid	M+H	355.102	-2.07395	249959.0449	6.6723
26	Cryptochlorogenic acid	M+H	355.102	-2.07395	249959.0449	6.6723
27	Resveratrol glycosides	M+H	355.102	-2.07395	249959.0449	6.6723
28	Myricetin	M+H	319.044	-1.46513	234391.1371	9.1317
29	Astragaloside	M-H	447.094	1.939	232758.5726	9.4846
30	Kaempferol 3-O-rutin	M-H	447.094	1.939	232758.5726	9.4846
31	Devasculin-3-glucoside	M-H	447.094	1.939	232758.5726	9.4846

32	5-(hydroxymethyl) -1H-pyrrole-2-formaldehyde	M-H	447.094	1.939	232758.5726	9.4846
33	Anthocyanins	M+H	305.101	-1.90299	110280.7613	12.658
34	Epigallocatechin	M+H	291.086	0.607	95307.367	10.4777
35	Isoquercetin	M-H	463.089	0.979	89105.35	7.7148
36	Isoquercitrin	M-H	463.089	0.979	89105.35	7.7148
37	Naringin	M-H	463.089	0.979	89105.35	7.7148
38	Kaempferol-3-O-β-D-glucopyranoside	M-H	319.047	2.165	81133.801	5.9919
39	5, 7-dihydroxychromogenone	M-H	389.125	1.846	60787.566	7.8139
40	Caffeic acid	M-H	179.0354	2.348	56059.463	1.6712