Electronic Supplementary Material (ESI) for Analytical Methods.

Supporting Information

Plasma metabolic profiling analysis of normal and ANIT-induced cholestasis rats after oral administration of Da-Huang-Xiao-Shi decoction by UHPLC-Q-Orbitrap MS coupled with pattern recognition

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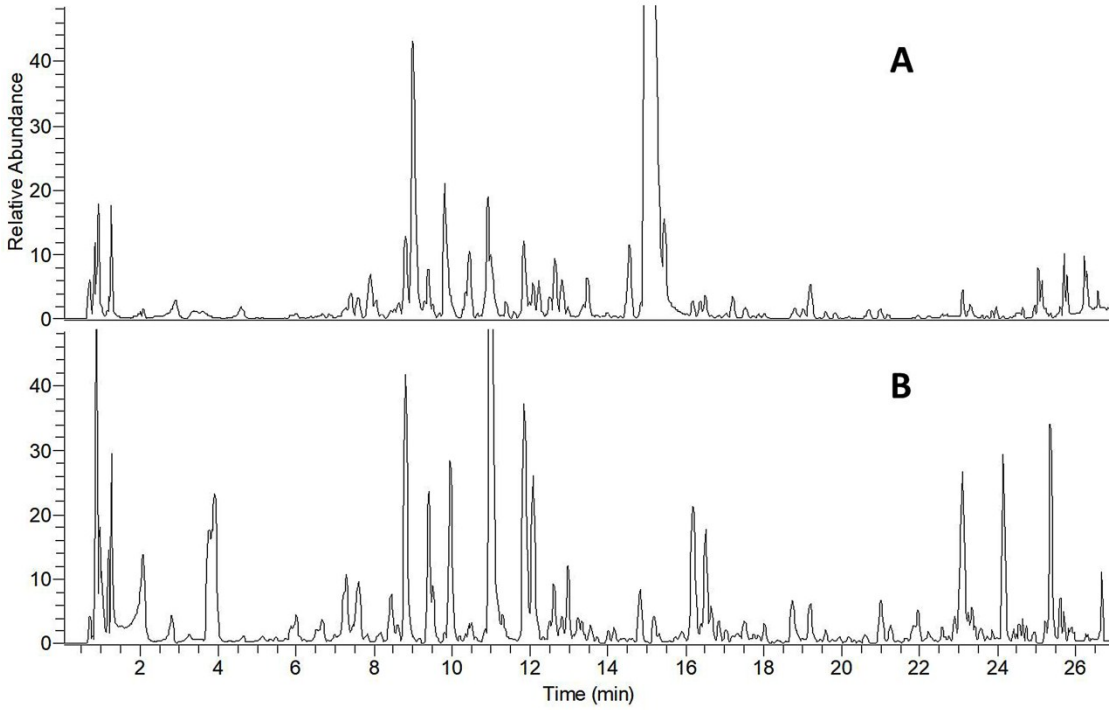

Fig.S1 Total ion chromatogram of DHXSD extract in positive-ion mode (A) and negative-ion mode (B)

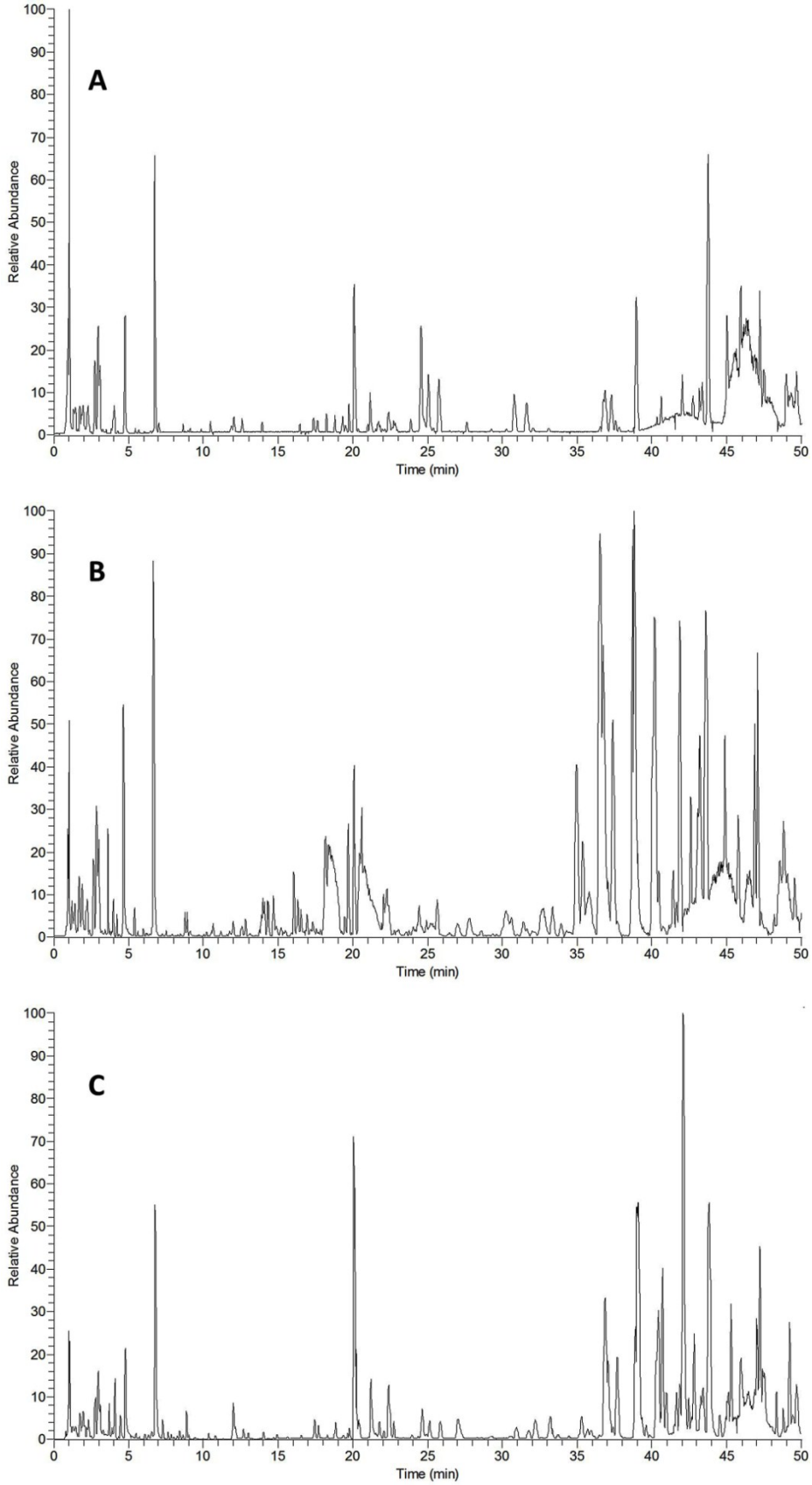

Fig.S2 The UHPLC-Q-Orbitrap MS total ion chromatograms of normal group, normal-DHXSD group and ANIT-DHXSD group plasma in positive mode.

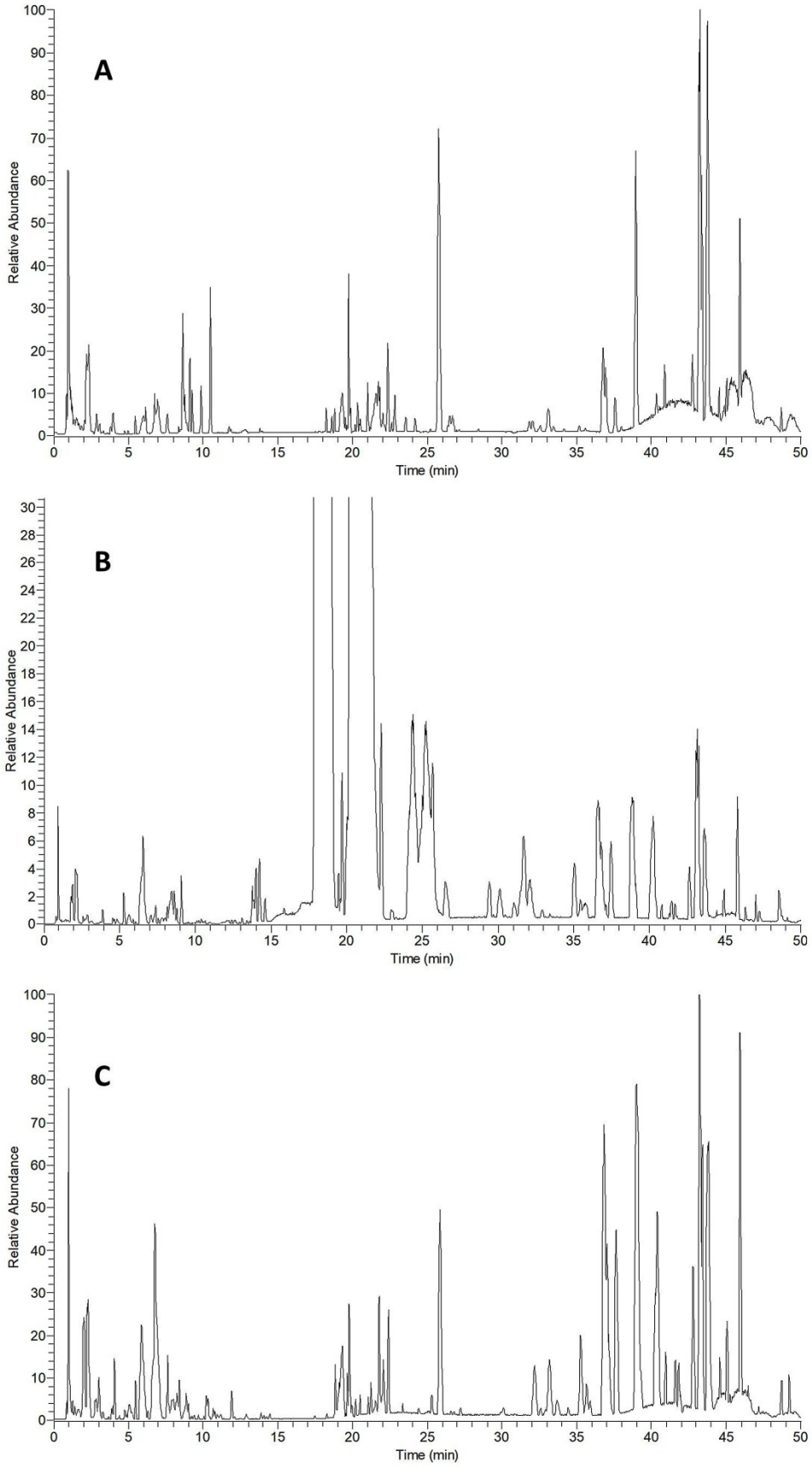

Fig.S3 The UHPLC-Q-Orbitrap MS total ion chromatograms of normal group, normal-DHXSD group and ANIT-DHXSD group plasma in negative mode.

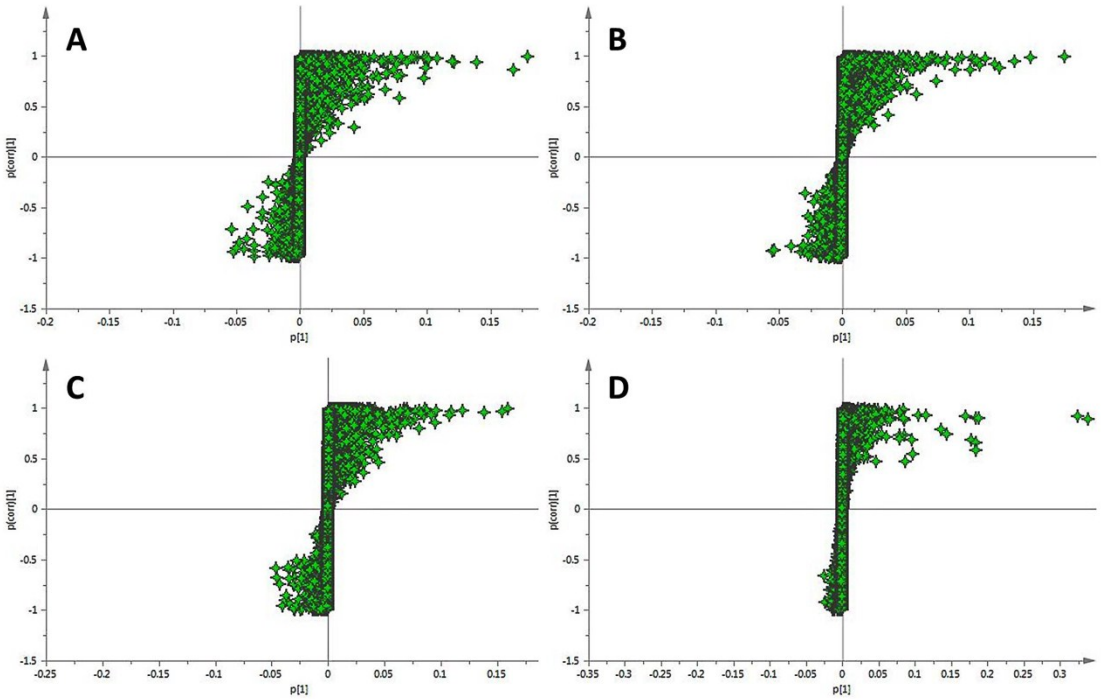

Fig.S4 S-plot of OPLS-DA model between normal group plasma samples and normal-DHXSD group plasma samples in positive ion mode (A) and negative ion mode (B); S-plot of OPLS-DA model between normal group plasma samples and ANIT-DHXSD group plasma samples in positive ion mode (C) and negative ion mode (D).

Table S1 Effect of ANIT on serum ALT, AST, ALP, TBIL, DBIL, and TBA, and γ-GT (n = 6, mean ± SD).

Groups	ALT (U/L)	AST (U/L)	ALP (U/L)	TBIL (μmoI/L)	DBIL(μmoI/L)	TBA (μmoI/L)	γ-GT（U/L）
Con	28.1037±4.9807	149.6701±18.2468	225.2167±27.8659	15.1608±2.4671	8.4601±2.7097	33.0127±14.8017	1.6571±0.8031
Mod	501.6801±51.6792***	890.3482±109.4793***	603.5777±79.5632**	187.3584±29.9641***	89.3571±19.3689**	168.9036±19.3702***	7.2803±1.1357**

Alanine transaminase (ALT); aspartate transaminase (AST); alkaline phosphatase (ALP); total bilirubin (TBIL); direct bilirubin (DBIL); total bile acid (TBA); and γ-glutamyltranspeptidase (γ-GT); **$p < 0.01$, ***$p < 0.001$ compared with control group.

Table S2 Indentification of prototype constituents of DHXSD in rat plasma by UHPLC-Q-Orbitrap MS under physiological and ANIT-induced cholestasis status.

NO.	t_R	Formula	Detected[M]	Diff	Accurate Weight	ESI^+/ ESI^-	Identification	Source
Positive								
P1	3.18	$C_{11}H_{18}ON$	180.1381	-3.886	180.1388	121.0646	Candicine	HB
P2	7.23	$C_{20}H_{24}O_4N$	342.1695	-2.923	342.1705	192.1017,177.0778	Phellodendrine	HB
P3	7.47	$C_{20}H_{26}O_4N$	344.1833	-8.475	344.1862	299.1258,175.0751	Tembetarine	HB
P4	7.81	$C_{20}H_{24}O_4N$	342.1695	-2.923	342.1705	297.1100,282.0867,265.0844, 237.0903, 192.0999	Magnoflorine	HB
P5	8.19	$C_{23}H_{30}O_8N$	448.1946	-6.038	447.1893	286.1419	N-Methylhigenamine-7-O-glucopyranoside	HB
P6	8.57	$C_{19}H_{24}NO_3$	314.1731	-2.865	314.1740	269.1157,192.1020,177.0901	Oblongine	HB
P7	8.59	$C_{21}H_{26}O_4N$	356.1834	-7.861	356.1862	311.1260,279.0998	Menisperine	HB
P8	9.45	$C_{19}H_{16}NO_4$	322.1207	-0.931	322.1210	307.0835, 292.1150	Berberrubine	HB
P9	9.71	$C_{20}H_{18}NO_5$	352.1197	-0.854	351.1120	337.0919,336.0847, 322.0692, 308.0896	Oxyberberine	HB
P10	11.30	$C_{20}H_{20}ON_4$	338.1365	-7.985	338.1392	323.1125,308.0897,294.1107,280.0922	Columbamine	HB
P11	11.90	$C_{20}H_{18}ON_4$	336.1209	-8.033	336.1236	321.0969,306.0739,292.0952,278.0798	Berberine	HB
P12	11.95	$C_{20}H_{20}ON_4$	338.1268	-1.183	338.1272	323.1125,308.0897,294.1107,280.0922	Jatrorrhizine	HB
P13	12.10	$C_{21}H_{22}ON_4$	352.1519	2.556	352.1510	337.1282,336.1209,322.1052,309.1294,308.1261	Palmatine	HB
Negative								
P14	2.18	$C_7H_{12}O_6$	191.0108	4.166	192.0180	173.0094,154.9989	Quinic acid	HB
P15	5.96	$C_{16}H_{24}O_{11}$	391.1238	-0.255	392.1319	229.0715,211.0615,185.0818,167.0713,149.0607	Shanzhiside	ZZ
P16	6.05	$C_{16}H_{22}O_{10}$	373.1130	-0.802	374.1213	211.0618,193.0506,167.0715,149.0607	Geniposidic acid	ZZ

P17	6.36	$C_{17}H_{24}O_{11}$	449.1298	0.742	404.1319	241.0715, 223.0610,101.0244	Scandoside methylester	ZZ
P18	6.40	$C_{17}H_{24}O_{11}$	449.1298	9.650	404.1319	241.0715,223.0610,205.0505,191.0347	Gardenoside	ZZ
P19	6.72	$C_{16}H_{26}O_{8}$	345.1552	1.156	346.1628	169.0926	Jasminoside B	ZZ
P20	6.82	$C_{30}H_{26}O_{12}$	577.1354	1.730	578.1424	425.0905,289.0710	Procyanidin B	ZZ
P21	7.36	$C_{10}H_{16}O_{3}$	183.0296	3.804	184.0369	168.0063,139.0036,124.0164	Jasminodiol	ZZ
P22	7.79	$C_{17}H_{20}O_{9}$	367.1033	-2.988	368.1102	193.0505,191.0560,173.0454,134.0374,111.0449	3-O-feruloylquinic acid	HB
P23	8.02	$C_{23}H_{34}O_{15}$	595.1880	1.091	550.1898	225.0768,207.0660,123.0451,101.0244	Genipin-1-β-D-gentiobioside	ZZ
P24	8.80	$C_{17}H_{24}O_{10}$	433.1337	7.214	388.1369	225.0769,207.0662,123.0450,101.0243	Geniposide	ZZ
P25	9.05	$C_{17}H_{20}O_{9}$	367.1033	-2.988	368.1102	193.0505,191.0566,175.0401,173.0464, 134.0374, 111.0450	5-O-feruloylquinic acid	HB
P26	9.49	$C_{16}H_{26}O_{7}$	375.1658	1.181	330.1679	167.0211,149.0457, 121.0295	Jasminoside A/E	ZZ
P27	9.69	$C_{16}H_{26}O_{7}$	375.1658	1.181	330.1679	167.0210,149.0455,121.0294	Jasminoside A/E	ZZ
P28	10.85	$C_{10}H_{16}O_{3}$	183.1025	3.259	184.1099	139.1128	Jasminodiol	ZZ
P29	11.18	$C_{15}H_{10}O_{5}$	269.0450	0.741	270.0528	241.04690,239.0337, 223.0399	Aloe-emodin	DH
P30	13.22	$C_{32}H_{40}O_{17}$	695.2208	-8.199	695.2265	225.0759,101.0243	6"-O-trans-feruloylgenipingentiobioside	ZZ
P31	16.62	$C_{31}H_{32}O_{16}$	659.1622	1.818	660.1690	497.1294,335.0748,161.0454	3,5-di-O-Caffeoyl-4-O-(3-hydroxy-3-methyl)-glutaroylquinic acid	ZZ
P32	17.32	$C_{15}H_{10}O_{4}$	253.0501	0.787	254.0579	225.0551,181.0209	Chrysophanol	DH
P33	21.45	$C_{15}H_{8}O_{6}$	283.0238	-1.056	284.0321	239.0359,211.0411,183.0459	Rhein	DH
P34	21.48	$C_{16}H_{12}O_{5}$	283.0618	4.576	284.0685	240.0382,239.0347,212.0440,211.0396	Physcion	DH
P35	25.16	$C_{15}H_{10}O_{5}$	269.0450	0.741	270.0528	241.0504,225.0555	Emodin	DH

Table S3 Indentification of metabolites of DHXSD in rat plasma by UHPLC-Q-Orbitrap MS under physiological and ANIT-induced cholestasis status.

NO.	t_R	Formula	Detected[M]	Diff (ppm)	Accurate Weight	ESI^+/ ESI^-	Identification	Source
Positive								
M1	4.26	$C_{11}H_{15}NO_3$	209.0912	-8.996	209.1052	193.0854,192.0653, 175.0854,163.0864	Genipinine	HB
M2	5.14	$C_{26}H_{32}NO_{10}$	518.2000	-5.017	518.2026	342.1703,192.1021,177.0787	Phellodendrine-O-glucuronide	HB
M3	5.17	$C_{24}H_{28}NO_9$	474.1743	-4.429	474.1764	298.1416, 283.1187	Hydromethyl armepavine-O-glucuronide	HB
M4	9.23	$C_{26}H_{26}NO_{13}S$	592.1172	7.938	592.1125	416.0843,336.1216, 321.1015, 306.0724,278.0849	Berberine-O-sulfate-O-glucuronide	HB
M5	9.39	$C_{25}H_{26}NO_{10}$	500.1542	-2.999	500.1557	324.1208, 309.0977,308.0906,280.0957	Demethyleneberberine-O-glucuronide	HB
M6	12.06	$C_{20}H_{20}NO_6$	370.1288	-0.811	370.1291	352.1162, 337.0952,308.0917, 293.1252	Dihydroxyberberine	HB
M7	12.79	$C_{19}H_{16}NO_6$	354.0950	1.694	354.0890	336.0844, 321.0604,306.0376,292.0587	Dihydroxyberberrubine	HB
M8	21.40	$C_{19}H_{18}NO_4$	324.1267	-0.926	324.1270	306.1104, 278.0800	Demethyleneberberine	HB
Negative								
M9	6.04	$C_{16}H_{22}O_{10}$	373.0920	5.880	374.1213	211.0613,193.0512,167.0714,158.0837,149.0607,123.0451	Geniposidic acid	ZZ
M10	7.09	$C_{16}H_{24}O_9$	359.1345	1.388	360.1420	341.1239,323.1127, 165.0917	Ring-opened and methyl formate removal derivative of genipin glucuronide	DH
M11	7.84	$C_{17}H_{20}O_{11}$	399.0930	1.000	400.1006	223.0609,208.0376,193.0140	Sinapoyl glucuronide	ZZ
M12	7.93	$C_{11}H_{14}O_8S$	305.0334	0.163	306.0409	225.0612,207.0509, 175.0611	Genipin-O-sulfate	ZZ
M13	8.07	$C_{11}H_{14}O_8S$	305.0348	6.208	306.0409	225.0765,207.0661,193.0505	Genipin-O-sulfate	ZZ

M14	8.78	$C_{17}H_{22}O_{11}$	401.1087	-0.497	402.1162	225.0764,207.0661,193.0354,175.0248, 123.0451,101.0244	Genipin-O-glucuronide	ZZ
M15	9.58	$C_{16}H_{24}O_9$	359.1346	0.167	360.1420	183.1025,165.0919	Ring-opened and methyl formate removal derivative of genipin glucuronide	DH
M16	9.80	$C_{16}H_{24}O_8$	343.1397	0.413	344.1471	167.0210,149.0245,131.0714	Ring-opened and reduced product of genipin-O-glucuronide	ZZ
M17	10.16	$C_{17}H_{22}O_{10}$	385.1143	2.590	386.1213	209.0665,193.0505,159.0295	Ring-opened dehydroxylation product of genipin-O-glucuronide	DH
M18	10.40	$C_{17}H_{20}O_{10}$	383.0981	1.302	384.1056	207.0509,189.0770,147.0452,119.0502	Dehydroxylation product of genipin-O-glucuronide	ZZ
M19	10.52	$C_{17}H_{20}O_{10}$	383.0982	1.562	384.1056	207.0661,192.0428	Dehydroxylation product of genipin-O-glucuronide	ZZ
M20	10.93	$C_{11}H_{14}O_5$	225.0614	-5.308	226.0841	207.0509,193.0504,175.0248	Genipin	ZZ
M21	11.04	$C_{15}H_{10}O_8S$	349.0024	2.286	350.0096	269.0447,241.1079,225.0616,213.1132	Emodin-O-sulfate	DH
M22	11.89	$C_{21}H_{16}O_{13}$	475.0662	2.353	476.0630	299.0199,271.0246,255.0292	Emodin acid monoglucuronide	DH
M23	11.91	$C_{28}H_{30}O_{16}$	621.1483	4.661	622.1534	283.0256,240.0744,212.0023	physcion-O-glucoside-O-glucuronide	DH
M24	12.69	$C_{27}H_{28}O_{15}$	591.1356	1.368	592.1355	253.0502,225.0564,182.0371	Chrysophanol-O-glucoside-O-glucuronide	DH
M25	13.09	$C_{21}H_{16}O_{12}$	459.0566	0.869	460.0642	283.0245,239.0347,255.0297,211.0400,183.0452	Rhein-O-glucuronide	DH
M26	15.58	$C_{18}H_{14}O_6$	325.0009	5.828	326.0070	282.9908,239.0003,221.9740	2-acetoxymethylchrysophanol	DH
M27	15.87	$C_{15}H_8SO_9$	362.9914	2.885	363.9889	283.0244,239.0346,211.0391,183.0455	Rhein-O-sulfate	DH
M28	17.26	$C_{21}H_{18}O_{10}$	429.0822	0.465	430.0900	253.0502,225.0546,113.0244	Chrysophanol-O-glucuronide	DH
M29	17.48	$C_{21}H_{18}O_{10}$	429.0825	-8.138	430.0940	253.0502,225.0546	Chrysophanol-O-glucuronide	DH
M30	17.85	$C_{21}H_{18}O_{11}$	445.0772	0.673	446.0849	269.0450,225.0551,113.0244	Emodin-O-glucuronide	DH

M31	19.26	$C_{21}H_{18}O_{11}$	445.0773	0.897	446.0849	269.0452,239.0356,211.0439	Aloe-emodin-O-glucuronide	DH
M32	21.25	$C_{23}H_{22}O_{11}$	473.0582	8.437	474.1162	297.0395,253.0499	laccaic acid D glucuronide	ZZ
M33	24.48	$C_{15}H_{10}O_6$	331.2257	-1.048	286.2320	285.2583,211.3235,195.1392	Hydroxyemodin	DH