Supporting information

Hepatotoxicity Assessment of Anshenbunao Syrup by Multi-Component Quantification In Vivo/In Vitro and Cell Biological Evaluations

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Table S1. The linear range, regression data and LLOQ for the determination of eleven characteristic components in ABS for their plasma exposure evaluation by UPLC-MS/MS.

Components	Regression equation	Linear range (ng/mL)	R^2	LLOQ (ng/mL)
IC	$Y=1.89\times10^{-2}X-5.36\times10^{-4}$	0.05-100	0.9921	0.05
BA	$Y=5.27\times10^{-2}X-5.02\times10^{-4}$	0.05-100	0.9909	0.05
TSG	$Y=4.43\times10^{-2}X-3.89\times10^{-4}$	0.05-100	0.9904	0.05
EP-C	$Y=5.34\times10^{-2}X-1.97\times10^{-3}$	0.05-100	0.9926	0.05
2'-O-RI II	$Y=1.53\times10^{-2}X-4.34\times10^{-4}$	0.05-100	0.9807	0.05
EP-B	$Y=3.83\times10^{-3}X-1.75\times10^{-3}$	0.20-100	0.9859	0.20
SA-B	$Y=1.87\times10^{-2}X-6.00\times10^{-4}$	0.05-100	0.9936	0.05
GTA	$Y=8.77\times10^{-3}X-1.11\times10^{-3}$	0.10-100	0.9891	0.10
LI	$Y=5.75\times10^{-2}X-7.27\times10^{-3}$	0.05-100	0.9907	0.05
EMG	$Y=1.29\times10^{-1}X-4.81\times10^{-4}$	0.05-100	0.9916	0.05
EM	$Y=3.40\times10^{-1}X-2.77\times10^{-1}$	0.02-100	0.9889	0.02

Table S2. Summary of method validation results for eleven key components of ABS in rats' plasma samples (n=6).

Components	Concentration	Intra-batch (n=6)		Inter-batch (n=3)		Matrix effect (%)	Extraction recovery (%)
	(ng/mL)	RSD (%)	RE (%)	RSD (%)	RE (%)	Mean±SD, RSD	Mean±SD, RSD
IC	0.1	9.68	-4.86	10.17	7.76	60.20±7.46,12.39	87.97±9.62,10.94
	2	9.42	-6.60	7.51	-6.93	63.14±4.87, 7.72	79.71±7.78, 9.76
	20	8.40	-9.61	8.46	-7.33	61.69±3.21, 5.20	71.93±4.53, 6.30
	80	6.18	-4.94	7.36	-7.38	69.52±5.39, 7.75	86.15±3.72, 4.32
BA	0.1	11.77	0.78	12.79	-2.37	54.92±3.22, 5.86	74.58±4.10, 5.50
	2	9.39	-6.52	10.38	-8.36	58.86±3.50, 5.94	79.75±7.12, 8.93
	20	9.53	-0.31	9.55	5.55	62.25±3.56, 5.73	69.32±6.17, 8.90
	80	6.43	-3.65	8.37	-6.61	79.23±3.22, 4.07	65.97±1.24, 1.88
TSG	0.1	9.59	8.94	8.56	9.96	64.56±2.99, 4.63	87.69±11.96,13.64
	2	8.07	4.54	8.77	7.14	72.54±2.28, 3.14	87.11±8.82,10.13
	20	8.24	-3.60	8.85	3.90	72.89±5.70, 7.82	81.80±6.10, 7.45
	80	8.18	-3.01	8.09	-3.39	67.74±5.30, 7.83	80.55±6.41, 7.95
EP-C	0.1	10.62	-3.14	9.75	5.53	64.95±7.31,11.26	63.91±4.15, 6.50
	2	8.71	5.05	9.40	-7.17	81.08±6.32, 7.79	91.14±4.25, 4.66
	20	9.76	-7.60	8.44	-6.76	55.47±4.80, 8.66	93.58±6.73, 7.19
	80	8.23	-8.77	8.81	-8.97	64.14±6.27, 9.77	98.91±5.93, 6.00
2'-O-RI Ⅱ	0.1	6.22	1.12	7.27	7.27	70.09±5.50, 7.84	69.30±8.59,12.39
	2	7.59	-7.57	9.66	-4.50	65.40±6.06, 9.26	78.37±3.80, 4.85
	20	5.69	-5.88	9.79	-1.94	55.26±4.48, 8.11	60.72±3.31, 5.45
	80	5.95	-4.05	9.11	-7.02	67.51±6.58, 9.74	72.17±6.51, 9.01
EP-B	0.1	11.66	-9.51	9.61	1.07	60.40±7.41,12.27	75.56±7.34, 9.71
	2	7.90	-7.37	9.23	-9.36	54.07±3.07,5.67	85.66±7.23, 8.45
	20	7.69	-9.81	8.68	-4.12	57.88±4.38, 7.57	81.53±3.89, 4.78
	80	7.61	-7.78	8.88	-8.19	55.98±2.64, 4.71	87.05±4.45, 5.11
SA-B	0.1	7.97	-7.09	9.52	-8.02	51.45±4.98, 9.68	81.96±4.65, 5.68
	2	8.76	-9.04	9.28	7.39	51.92±3.13, 6.04	79.19±5.27, 6.65
	20	8.53	9.59	8.60	9.49	51.84±4.45, 8.58	75.72±4.50, 5.94
	80	6.66	-5.76	4.87	-6.93	61.26±2.80, 4.57	96.50±9.56, 9.91
GTA	0.1	10.28	-7.00	10.32	-8.96	77.19±7.65, 9.92	90.61±8.46, 9.34
	2	9.97	8.42	9.16	7.49	59.46±5.59, 9.40	81.92±7.81, 9.53
	20	9.71	-4.62	8.78	7.59	55.30±2.26, 4.09	76.82±5.87, 7.64
	80	9.63	-4.67	3.04	4.07	58.61±3.13, 5.34	83.24±3.49, 4.20
LI	0.1	8.08	7.83	9.61	-5.31	59.17±2.72, 4.59	70.27±10.13,14.41
	2	9.95	-5.50	10.65	1.40	63.61±5.16, 8.12	91.10±8.91, 9.78
	20	8.86	-5.78	9.08	-2.89	54.56±4.30, 7.89	73.05±7.22, 9.88
	80	5.47	-5.26	6.41	-7.03	56.54±5.59, 9.88	107.37±9.91, 9.23

EMG	0.1	11.75	0.16	11.57	-3.24	53.95±3.76, 6.96	82.09±8.83,10.76
	2	11.03	7.42	12.30	6.60	61.43±5.70, 9.29	82.66±2.36, 2.86
	20	12.99	-7.52	12.31	-7.76	49.92±4.27, 8.55	74.69±2.07, 2.78
	80	10.78	-3.66	11.62	-7.82	60.65±5.20, 8.57	86.45±3.17, 3.67
EM	0.1	13.71	0.11	12.98	-9.70	79.67±3.92, 4.92	82.82±9.86,11.90
	2	12.96	-2.80	12.85	-6.01	79.72±4.99, 6.26	85.58±8.03, 9.39
	20	7.97	-4.01	12.53	-7.47	79.75±7.50, 9.41	78.61±1.77, 2.25
	80	8.49	-6.04	9.16	-6.33	61.86±4.97, 8.03	88.76±7.33, 8.25

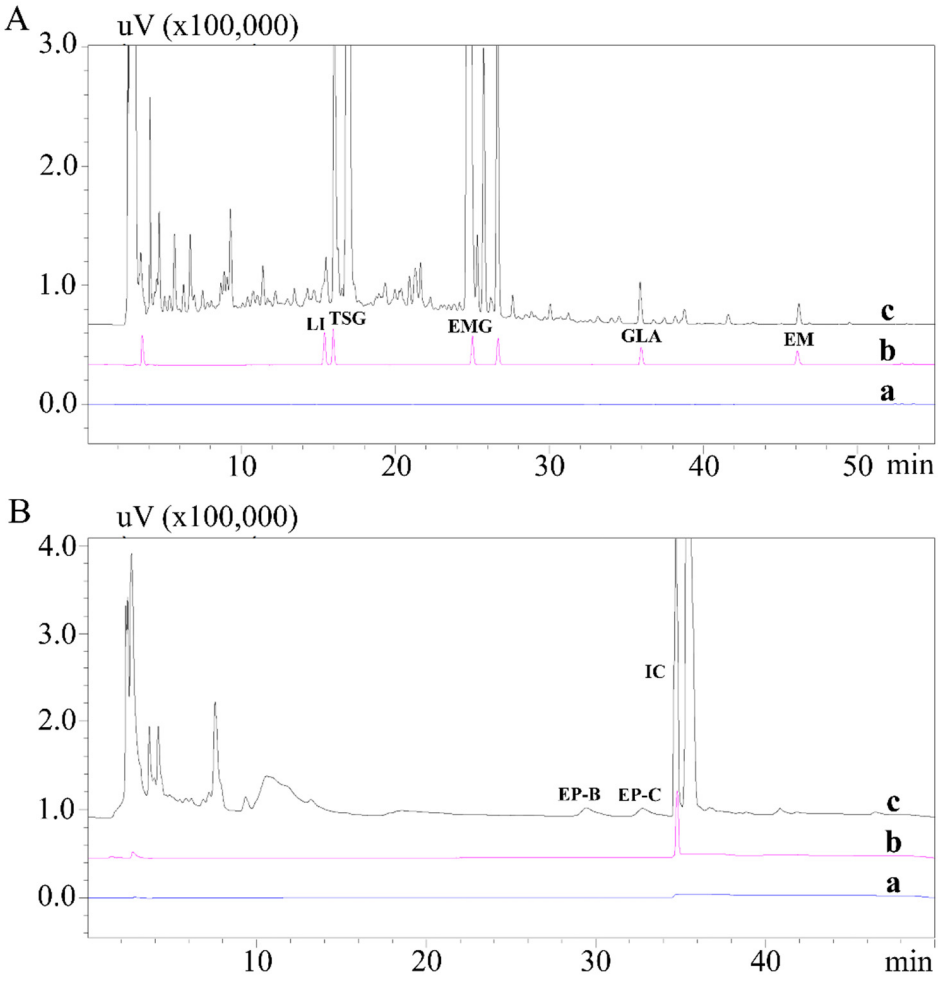


Figure S1. HPLC–UV chromatograms of eight key components determined in the ABS. (**A**). external standard method: a. blank, b. reference mixture containing 10 μg/mL LI, 10 μg/mL TSG, 2 μg/mL EMG, 8 μg/mL GLA, and 2 μg/mL EM, c. commercially available ABS. (**B**). quantitative analysis of multi–components by the single–marker (QAMS) method: a. blank, b. reference of 40 μg/mL IC, c. commercially available ABS.

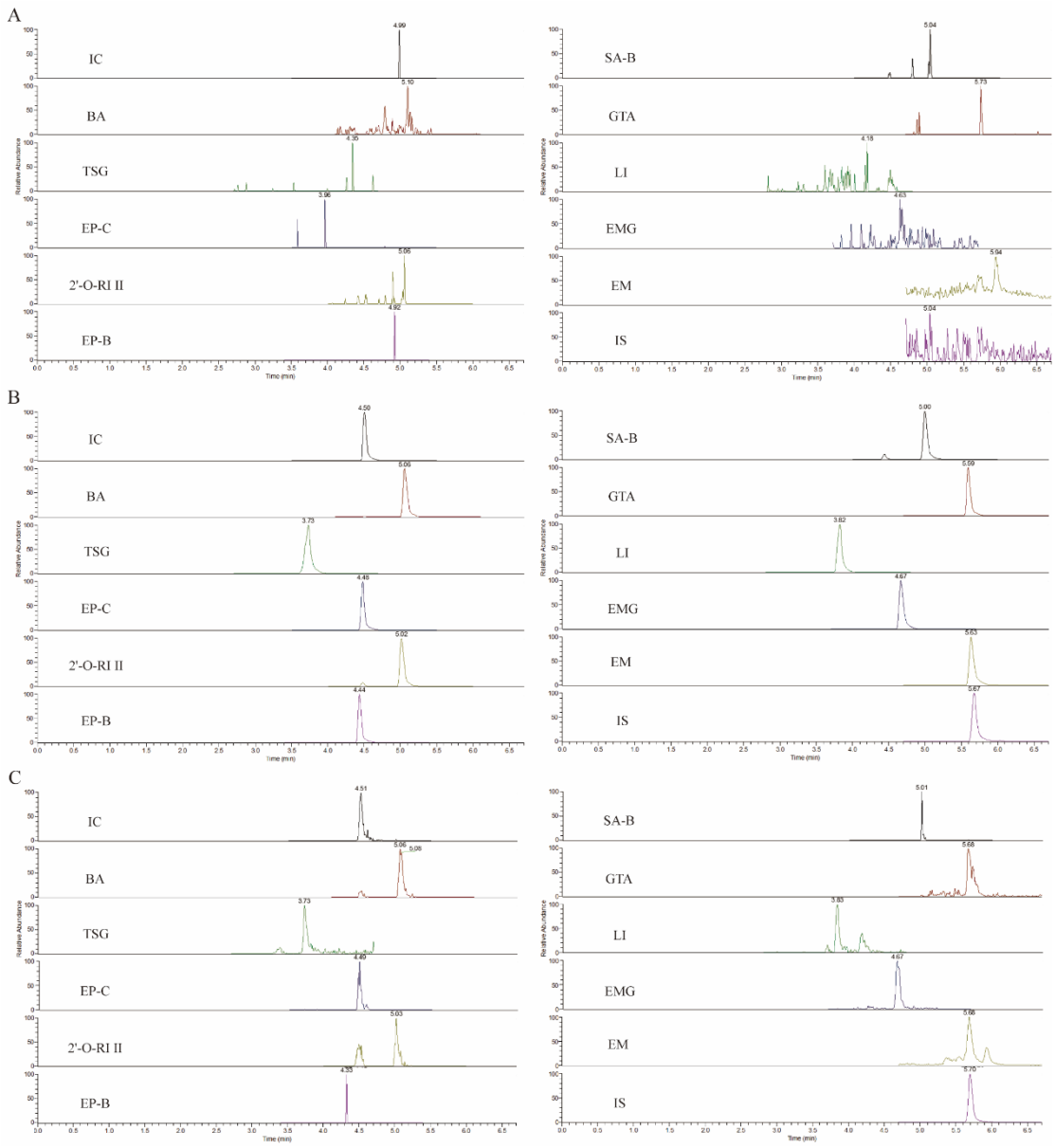


Figure S2. UPLC–MS/MS chromatograms of eleven components determined in the rats' plasma samples. (**A**) blank plasma. (**B**) blank plasma spiked with IC, BA, TSG, EP-C, 2'-O-RI II, EP-B, SA-B, GTA, LI, EMG, EM, and IS. (**C**) plasma samples obtained from rats after oral administration of ABS.

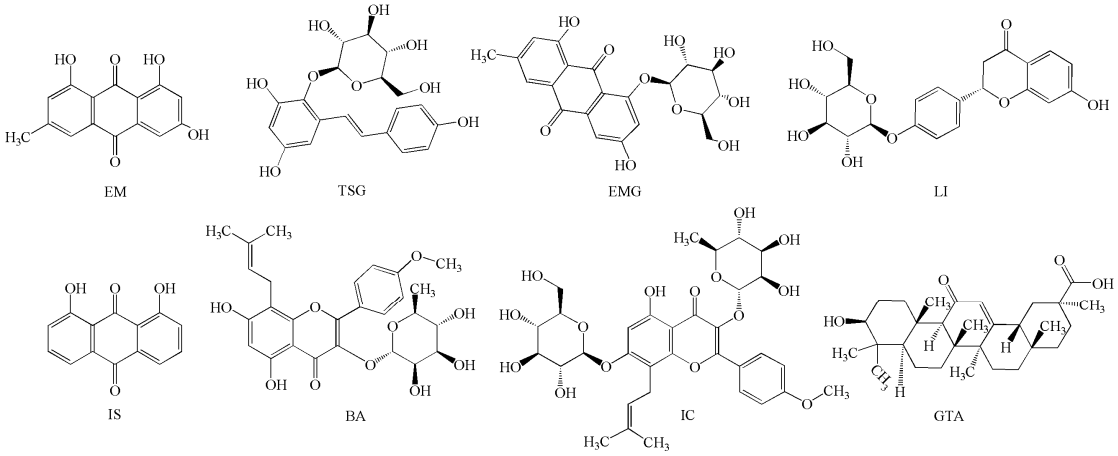


Figure S3. Chemical structures of the quality marker components in ABS.

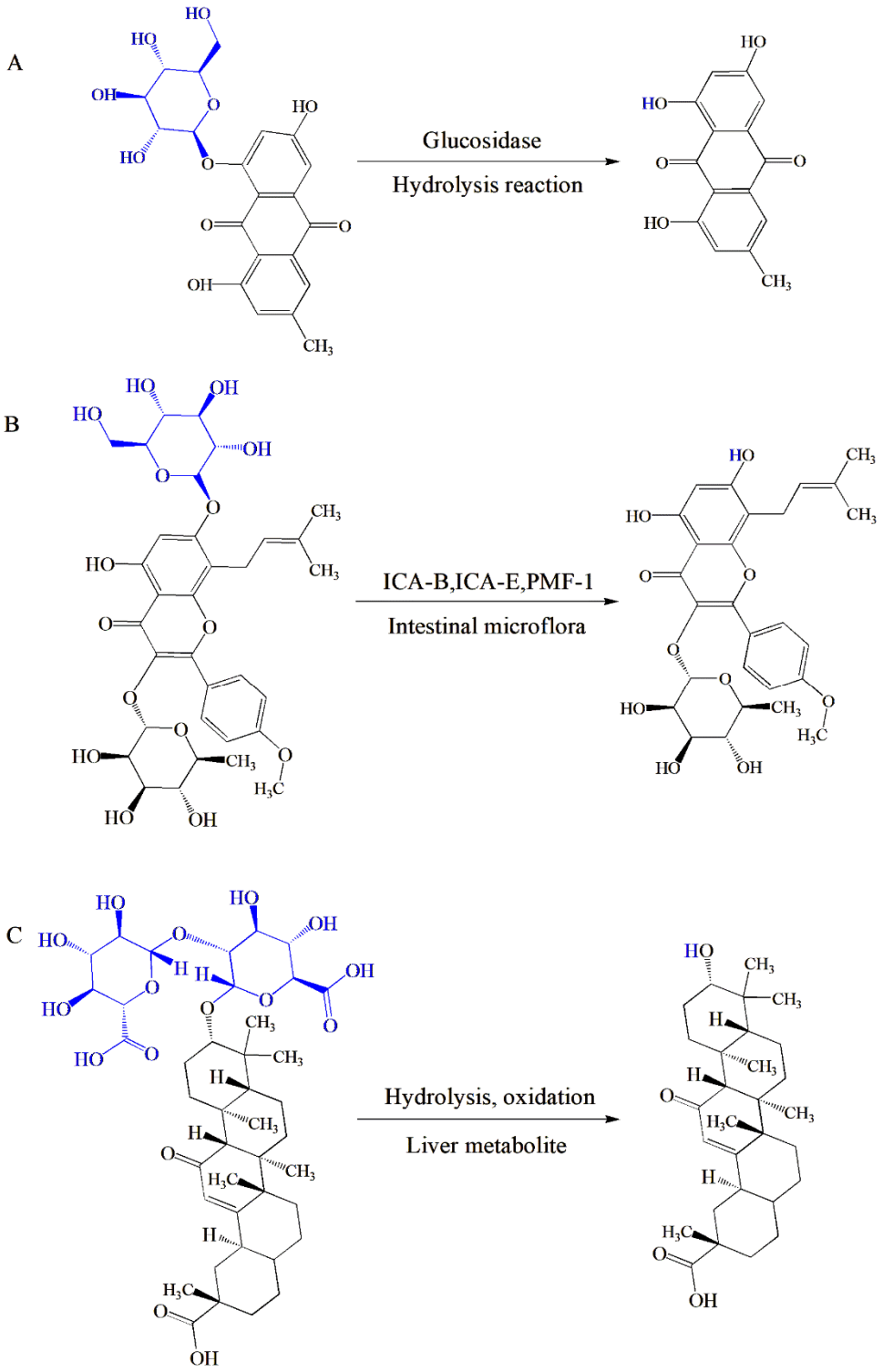


Figure S4. The primary metabolic pathways and corresponding metabolites of the three glucoside types or their analogs. (**A**) EMG. (**B**) IC. (**C**) GLA.