Electronic Supplementary Information

Selective separation and identification of metabolite groups of *Polygonum cuspidatum* extract in rat plasma using dispersion solid-phase extraction by magnetic molecularly imprinted polymers coupled with LC/Q-TOF-MS

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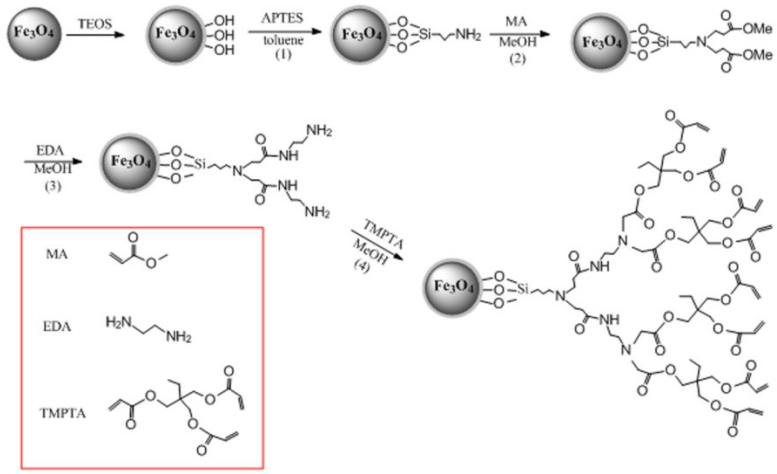

Fig. S1 Schematic representation of dendritic-grafting modification of Fe_3O_4@SiO_2 MNPs.

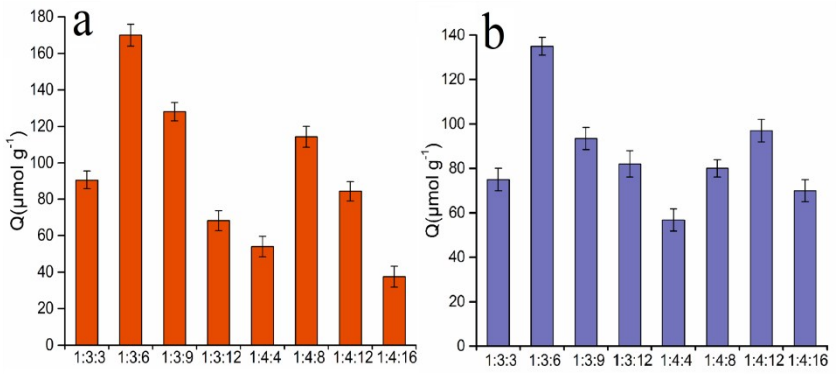

Fig. S2 (a) Affinity for polydatin of PD-MMIPs with different molar ratios of template (polydatin): monomer (MAA): cross linker (EGDMA) (b) Affinity for emodin-8-*O*-*β*-D-glucoside of EG-MMIPs with different molar ratios of template (emodin-8-*O*-*β*-D-glucoside): monomer (MAA): cross linker (EGDMA).

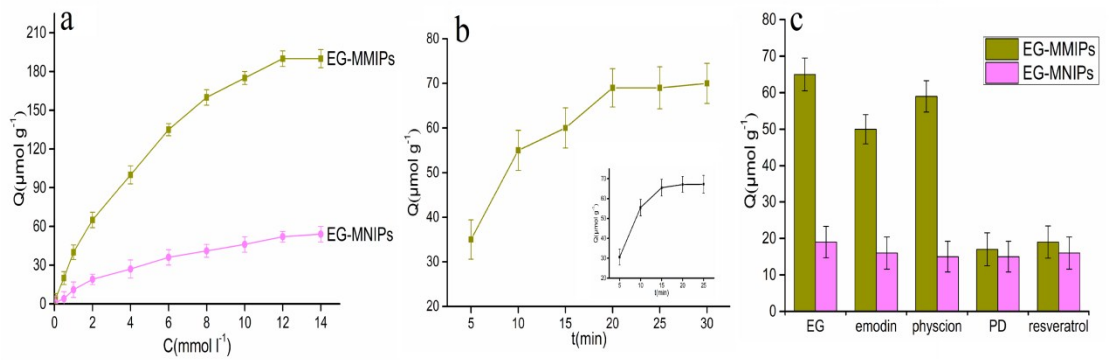

Fig. S3 (a) Adsorption isotherm of EG-MMIPs and EG-MNIPs; (b) Adsorption and desorption kinetic curves of EG-MMIPs; (c) Selective recognition property of each compound with EG-MMIPs and EG-MNIPs at the concentration of 2mmol l^{-1}.

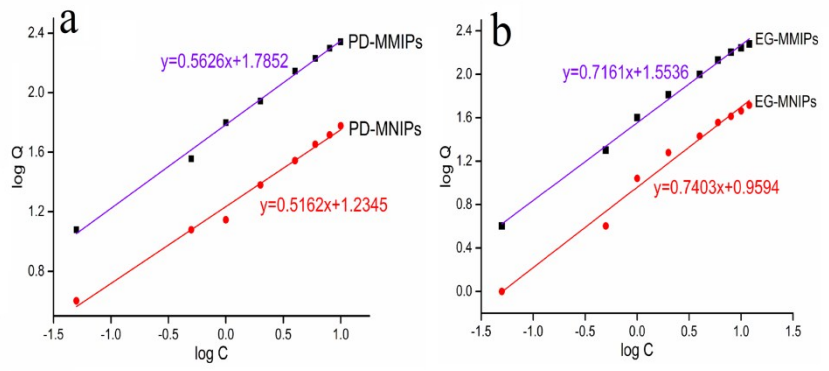

Fig. S4 Fitting plots with Freundlich isotherm model (a) PD-MMIPs and PD-MNIPs (b) EG-MMIPs and EG-MNIPs.

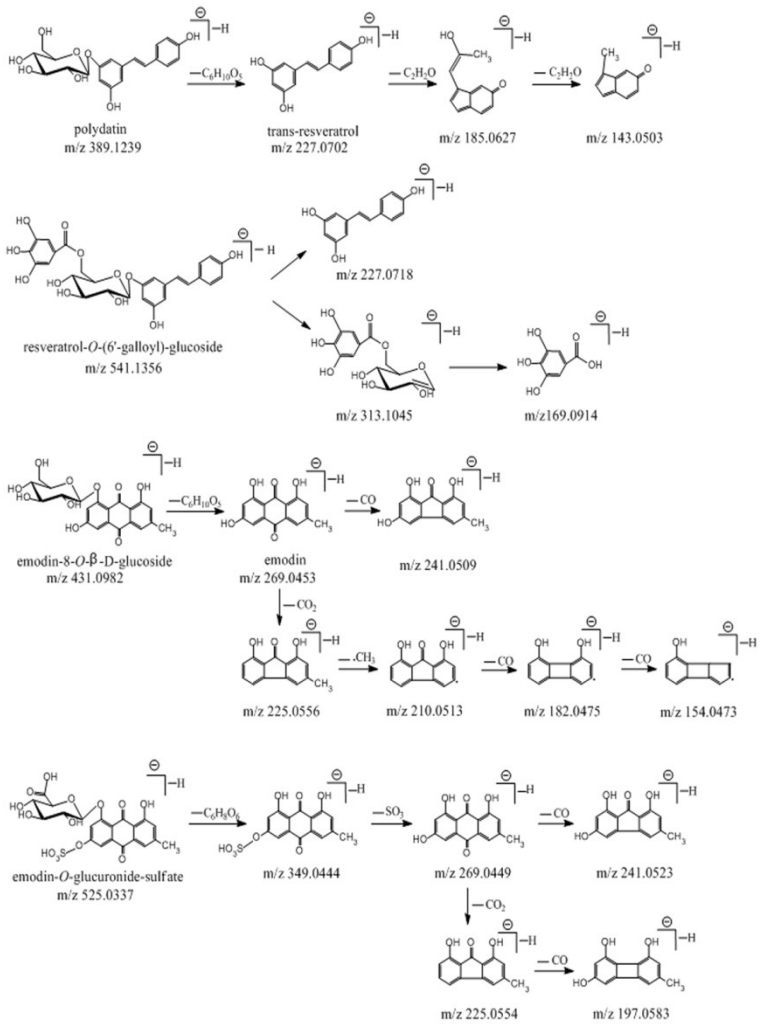

Fig. S5 The proposal fragmentation pathways of reference standards (polydatin and emodin-8-*O*-*β*-*D*-glucoside) and metabolites (S16: resveratrol-*O*-(6'-galloyl)-glucoside and A1: emodin-*O*-glucuronide sulfate).

Table S1. $\Delta E_{binding}$ of complexes between template and monomer

molecules	PD-MMIPs			EG-MMIPs		
	energy (a. u.)	ΔE (a. u.)	ΔE (kJ mol^{-1})	energy (a. u.)	ΔE (a. u.)	ΔE (kJ mol^{-1})
PD	-1369.5214					
EG	-1555.8604					
MAA	-304.7912					
TFMAA	-600.8918					
AA	-265.6838					
PD/EG:MAA	-1674.3529	0.0404	105.9256	-1860.6929	0.0413	108.3267
PD/EG:2MAA	-1979.1878	0.0840	220.5322	-2165.5449	0.1021	267.9738
PD/EG:3MAA	-2284.0211	0.1262	331.2208	-2470.3859	0.1519	398.7281
PD/EG:4MAA	-2588.8460	0.1599	419.6026	-2775.2272	0.2021	530.3907
PD/EG:5MAA	-2893.2947	0.1827	479.5466	-3079.6009	0.2155	565.7595
PD/EG:TFMAA	-1970.4516	0.0385	100.9605	-2156.7907	0.0385	101.0566
PD/EG:2TFMAA	-2571.3820	0.0771	202.3723	-2757.7443	0.1004	263.4374
PD/EG:3TFMAA	-3172.3141	0.1175	308.3449	-3358.3858	0.1500	393.7433
PD/EG:AA	-1635.2448	0.0397	104.2436	-1821.5849	0.0408	107.0302
PD/EG:2AA	-1900.9676	0.0787	206.5921	-2087.3290	0.1011	265.4702
PD/EG:3AA	-2166.6922	0.1196	313.9513	-2353.0632	0.1515	397.7419

Table S2. $\Delta E_{solvation}$ in different solvents

solvents	PD-MMIPs		EG-MMIPs	
	ΔE (a. u.)	ΔE (kJ mol^{-1})	ΔE (a. u.)	ΔE (kJ mol^{-1})
acetone	0.02455	64.4508	0.03148	82.6223
toluene	0.01342	35.2177	0.01472	38.6441
methanol	0.02920	76.6440	0.03258	85.5232
tetrahydrofuran	0.02408	63.2018	0.02671	70.1204
acetonitrile	0.02934	77.0209	0.03274	85.9542

3.5 *Identification of different metabolite groups in rat plasma using LC/Q-TOF-MS*

3.5.1 *Identification of stilbenoid metabolite groups*

Compound S1-S18 showed similar fragmentation pattern which were typical of resveratrol and its derivatives. S15 showed identical RT and MS behaviors with reference standard (Fig. S5) and was unequivocally identified as trans-resveratrol. S5 (RT = 19.5 min), S10 (RT = 24.1 min) and S11 (RT = 24.4 min) generated high-intensity ions at m/z 389, 227 and 185, representing $[M-H]^-$, $[M-H-C_6H_{10}O_5]^-$ and $[M-H-C_6H_{10}O_5-C_2H_2O]^-$, respectively, which was corresponding to the loss data of reference substance PD. By comparison with RT, S5 was definitely identified as PD, and S10 and S11 were tentatively assigned as isomers of resveratrol-*O*-glucoside I and II.

S1 displayed a high resolution $[M-H]^-$ ion at m/z 565.1545 and gave element composition of $C_{26}H_{29}O_{14}$. The MS^2 spectrum gave a dominant ion at m/z 389.1539 after lost a glucuronide (176 Da) and produced fragment ion at m/z 227.0759 in MS^3 spectra. S1 was characterized as polydatin-*O*-glucuronide.

S2 and S3 both gave the precursor ions $[M-H]^-$ at m/z 469. The consecutive neutral loss of 80 Da and 162 Da forming ions at m/z 389 and 227 in MS^n spectra indicated the presence of a sulfate unit and a glucose unit. S2 and S3 were tentatively assigned as trans-polydatin sulfate and cis-polydatin sulfate according to the retention time in reference.[1]

S4 and S14 produced the $[M-H]^-$ ion at m/z 403, and further exhibited $[M-H-C_6H_8O_6]^-$ and $[M-H-C_6H_8O_6-C_2H_2O]^-$ ions at m/z 227 and 185, respectively,

indicating that they possessed the same aglycone resveratrol. Furthermore, corresponding to the loss of 176 Da, S4 and S14 were identified as resveratrol-*O*-glucuronide I and II.[2]

S7 (RT = 21.8 min), S8 (RT = 23.4 min) and S13 (RT = 26.6 min) exhibited identical molecular $[M-H]^-$ and product ions which indicated they were isomers. They obtained the precursor ions $[M-H]^-$ at m/z 307, then generated the fragment ion at m/z 227 in MS^2 spectra, representing the loss of a sulfate (80 Da). Furthermore, the product ions at m/z 227, 185 and 143 suggested that they possess the resveratrol aglycone. Therefore, S7, S8 and S13 were assigned as resveratrol sulfate I, II and III.[3]

S16 gave a deprotonated ion $[M-H]^-$ at m/z 541.1356, and further exhibited ions at m/z 313.1045, 227.0718 and 169.0914, indicating a galloyl unit and a glucoside unit. S16 was tentatively deduced as resveratrol-*O*-(6'-galloyl)-glucoside[4] and the proposal fragmentation pathway was shown in Fig. S5.

S6 produced the $[M-H]^-$ ion at m/z 309.0435 ($C_{14}H_{13}O_6S$), and yielded product ions at m/z 229.0460, 187.0217 and 145.0037, corresponding to the losses of a sulfate unit (80 Da) and two C_2H_2O groups (42 Da). The aglycone ion at m/z 229.0860 ($C_{14}H_{13}O_3$) was 2 Da higher than that of resveratrol, implying that there were two more hydrogen in this aglycone. Thus, S6 was characterized as 7, 8-dihydro-resveratrol sulfate.[5]

S9 and S12 exhibiting $[M+HCOOH-H]^-$ at m/z 467 and $[M-H]^-$ at m/z 421, showed the elemental composition of $C_{20}H_{21}O_{10}$ ($[M-H]^-$). They further yielded the prominent ion at m/z 259, which referred to a glucose loss (162 Da). According to the

reference,[6] S9 and S12 were identified as pentahydroxystilbene-*O*-glucoside I and II.

S17 gave the $[M-H]^-$ ion at m/z 447.1285 ($C_{22}H_{23}O_{10}$), then produced ion at m/z 243.1361 ($C_{14}H_{11}O_4$), representing the loss of a glucoside unit and a acetyl unit. The other two prominent ions at m/z 225.1583 and 215.1191 were assigned as loss H_2O and CO in benzene ring after rearrangement, respectively. Thus S17 was preliminarily assigned as tetrahydroxystilbene-*O*-(acetyl)-glucoside.[6]

S18 gave a $[M-H]^-$ ion at m/z 405.1206 ($C_{20}H_{21}O_9$). The product ion at m/z 243.1290 originated from the loss of a glucoside unit (162 Da). The further fragmentation was identical with S17. Therefore S18 was identified as tetrahydroxystilbene-*O*-glucoside according to the literature.[6]

3.5.2 *Identification of anthraquinone metabolite groups*

Based on MS^n spectra, 20 anthraquinone derivatives (compounds A1-A20) were rapid identified. By comparing RT and mass spectra with standards, A3, A13 and A20 were identified as EG, aloe-emodin and emodin, respectively. The fragmentation pathways of standards EG and emodin were explained in Fig. S5. A17 showed the same precursor ion and fragment ions with EG, which indicated A17 was emodin-*O*-glucoside.

A1, A2, A4, A7, A8, A10, A12, A18 and A19 showed dominant ions at m/z 269 with the further released fragment ions at m/z 241 or 225, these compounds were assigned as emodin derivatives.

A1 produced a precursor ion at m/z 525.0337 and yielded ions at m/z 349.0444 and 269.0449, which referred to a glucuronide (176 Da) and a sulfate (80 Da) loss. So

A1 was tentatively characterized as emodin-*O*-glucuronide sulfate.[7] The possible fragmentation pathway of A1 was shown in Fig. S5.

A2 (RT = 27.8 min), A8 (RT = 35.2 min) and A12 (RT = 38.2 min) had same $[M-H]^-$ ion at m/z 445 ($C_{21}H_{17}O_{11}$). The consecutive neutral loss of 176 Da forming ion at m/z 269 in MS^2 spectra suggested they were emodin-*O*-glucuronide isomer I, II and III.[8]

A4, A10 and A18 generated fragment ions at m/z 349 and 269, representing $[M-H]^-$, $[M-H-SO_3]^-$, respectively. Thus A4, A10 and A18 were tentatively identified as emodin sulfate I, II and III.[8]

A7 and A19 obtained the $[M-H]^-$ ion at m/z 313 ($C_{16}H_9O_7$) and then produced the characteristic fragment ion at m/z 269, corresponding to the loss of CO_2. Therefore A7 and A19 were proposed as carboxyl emodin I and II.[6]

A5 (RT = 32.8 min), A9 (RT = 36.3 min) and A15 (RT = 45.8 min) showed a dominant ion at 283 ($C_{16}H_{12}O_5$) in MS^2 spectrum, and the consecutive losses of CO (28 Da) or $\bullet CH_3$ (15 Da), which indicated the existence of physcion aglycone according to the reference.[9] A5 produced the deprotonated ion $[M-H]^-$ at m/z 445.1131 ($C_{22}H_{21}O_{10}$) and m/z 283.0834 ($C_{16}H_{12}O_5$), corresponding to the loss of a glucose (162 Da). Thus A5 was tentatively identified as physcion-*O*-glucoside. A9 and A15 generated the identical $[M-H]^-$ ion at m/z 459 ($C_{22}H_{19}O_{11}$) and the same fragment ion at m/z 283 after the neutral loss of 176 Da. Hence, they were tentatively assigned as physcion-*O*-glucuronide I and II.

A16 gave the $[M-H]^-$ ion at m/z 429.0818 ($C_{21}H_{17}O_{10}$) and the product ion at m/z

253.1857 ($C_{15}H_9O_4$), indicating the loss of 176 Da ($C_6H_8O_6$). Further product ions at m/z 225.1760, 210.1969, 182.9779 indicated that the aglycone was chrysophanol.[10] Thus A16 was tentatively characterized as chrysophanol-*O*-glucuronide.

A6 and A11 produced the same $[M-H]^-$ ion at m/z 285, and then A6 yielded product ions at m/z 268.0400, 257.0706 and 255.0517, and A11 obtained ions at m/z 267.0566 and 241.0485. By compared with reference, A6 and A11 were identified as ω-hydroxyemodin and hydroxyemodin, respectively.[11]

The protonated ion of A14 was exhibited at m/z 445.0771 ($C_{21}H_{17}O_{11}$), and the further fragment ions were at m/z 283.0638. According to the reference, A14 was tentatively assigned as rhein-*O*-glucoside.[12]

3.5.3 *Identification of other compounds*

Besides, five other compounds were identified in different pretreatment methods. C1-C5 were tentatively identified as catechin-*O*-hex, torachryson-*O*-glucoside, quercetin-*O*-glucoside, torachrysone-*O*-(6'-acetyl)-glucoside and quercetin, respectively.[6, 13]

References

1 R. Moss, Q. Mao, D. Taylor and C. Saucier, Rapid Commun. Mass Spectrom., 2013 27, 1815-1827.

2 M. Muzzio, Z. Huang, S. C. Hu, W. D. Johnson, D. L. McCormick and I. M. Kapetanovic, J. Pharm. Biomed. Anal., 2012, 59, 201-208.

3 M. Urpi-Sarda, R. Zamora-Ros, R. Lamuela-Raventos, A. Cherubini, O. Jauregui, R. de la Torre, M. I. Covas, R. Estruch, W. Jaeger and C. Andres-Lacueva, Clin. Chem., 2007, 53, 292-299.

4 D. Tang, J. X. Zhu, A. G. Wu, Y. H. Xu, T. T. Duan, Z. G. Zheng, R. S. Wang, D. Li and Q. Zhu, J. Chromatogr. A, 2013, 1286, 102-110.

5 M. Azorín-Ortuño, M. J. Yáñez-Gascón, F. Vallejo, F. J. Pallarés, M. Larrosa, R. Lucas, J. C. Morales, F. A. Tomás-Barberán, M. T. García-Conesa and J. C. Espín, Mol. Nutr. Food Res., 2011, 55, 1154-1168.

6 X. H. Qiu, J. Zhang, Z. H. Huang, D. Y. Zhu and W. Xu, J. Chromatogr. A, 2013, 1292, 121-131.

7 R. Song, L. Xu, F. G. Xu, Z. Li, H. J. Dong, Y. Tian and Z. J. Zhang, J. Chromatogr. A, 2010, 1217, 7144-7152.

8 H. Y. Zhu, K. S. Bi, F. Han, J. Guan, X. S. Zhang, X. J. Mao, L. S. Zhao, Q. Li, X. H. Hou and R. Yin, J. Pharm. Biomed. Anal., 2015, 111, 277-287.

9 E. Varga, T. Glauner, F. Berthiller, R. Krska, R. Schuhmacher and M. Sulyok, Anal. Bioanal. Chem., 2013, 405, 5087-5104.

10 W. Cao, L. Yi, L. H. Ye, J. Cao, S. S. Hu, J. J. Xu, L. Q. Peng, Q. Y. Zhu and Q. Y.

Zhang, Electrophoresis, 2015, 36, 2404-2412.

11 R. Song, H. Lin, Z. J. Zhang, Z. Li, L. Xu, H. J. Dong and Y. Tian, Rapid Commun. Mass Spectrom., 2009, 23, 537-547.

12 M. A. Farag, A. Porzel, E. A. Mahrous, M. M. El-Massry and L. A. Wessjohann, Anal. Bioanal. Chem., 2015, 407, 1937-1949.

13 F. Khallouki, R. Haubner, I. Ricarte, G. Erben, K. Klika, C. M. Ulrich and R. W. Owen, Food Chem., 2015, 179, 191-198.