Electronic Supplementary Material (ESI) for Analyst.

Supporting Information

Submicron 3,4-Dihydroxybenzoic acid-TiO_2 Composite Particles for Enhanced MALDI MS Imaging of Secondary Metabolites in the Root of Different-aged Baical Skullcap

Ruiyang Sun†, Ying Zhang†, Weiwei Tang, Bin Li*

State Key Laboratory of Natural Medicines and School of Traditional Chinese Pharmacy, China Pharmaceutical University, Nanjing, 210009, China

---

CONTENTS

Figure S1. Coordination structures for A) catecholate and B) salicylate binding.

Figure S2. MALDI mass spectra acquired from root sections of *S. baicalensis* using A) 2,3-DHB-TiO_2 CPs, B) 2,4-DHB-TiO_2 CPs, C) 2,5-DHB-TiO_2 CPs, D) 2,6-DHB-TiO_2 CPs, and E) 3,5-DHB-TiO_2 CPs, respectively.

Figure S3. Comparison of the mean peak intensities of five representative flavonoids measured using six different DHB-TiO_2 CPs.

Figure S4. MALDI mass spectra acquired from root sections of *S. baicalensis* using A) DHB and B) CHCA in the positive ion mode, respectively. Background MALDI mass spectra obtained by using C) DHB and D) CHCA in the positive ion mode, respectively.

Table S1. Metabolite putatively assigned in *S. baicalensis* root tissue by positive ion MALDI MS using 3,4-DHB-TiO_2 CPs.

Table S2. Determination of major metabolites in *S. baicalensis* root tissues by LC-MS/MS.

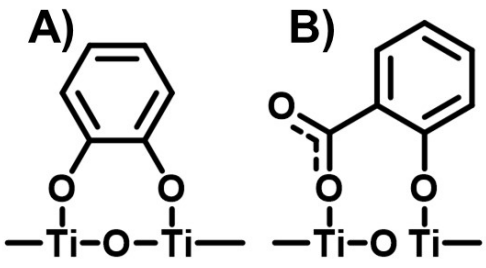

Figure S1. Coordination structures for A) catecholate and B) salicylate binding.

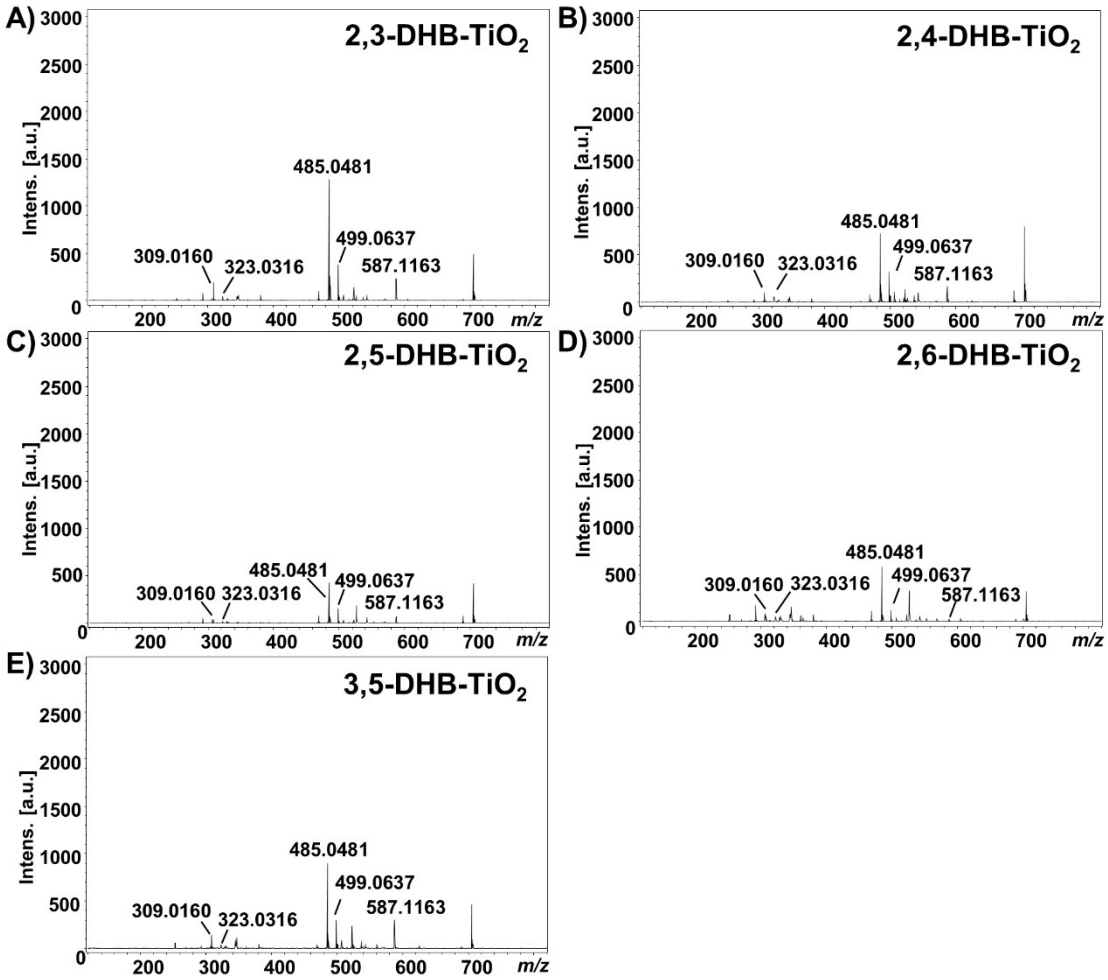

Figure S2. MALDI mass spectra acquired from root sections of *S. baicalensis* using A) 2,3-DHB-TiO_2 CPs, B) 2,4-DHB-TiO_2 CPs, C) 2,5-DHB-TiO_2 CPs, D) 2,6-DHB-TiO_2 CPs, and E) 3,5-DHB-TiO_2 CPs, respectively.

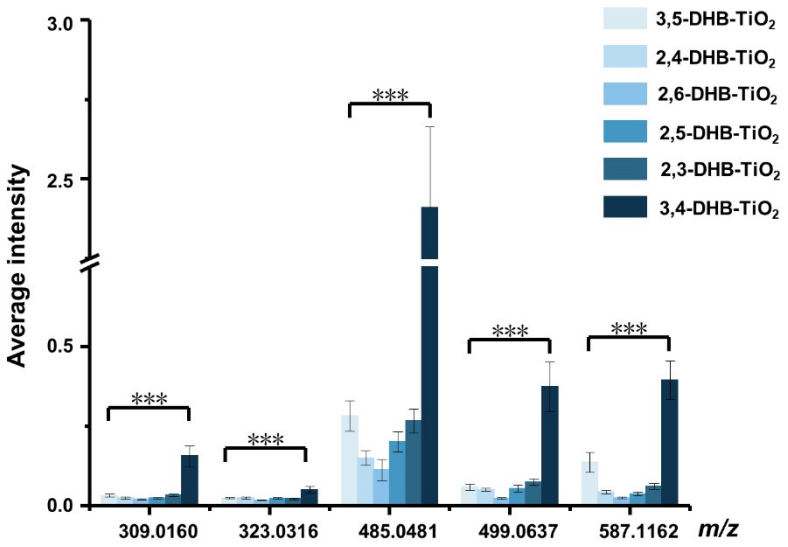

Figure S3. Comparison of the mean peak intensities of five representative flavonoids measured using six different DHB-TiO_2 CPs. Data are expressed as mean ± SD (n=10), and One-way ANOVA statistical analysis was performed for each group, significantly different from 3,4-DHB-TiO_2 CPs at $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

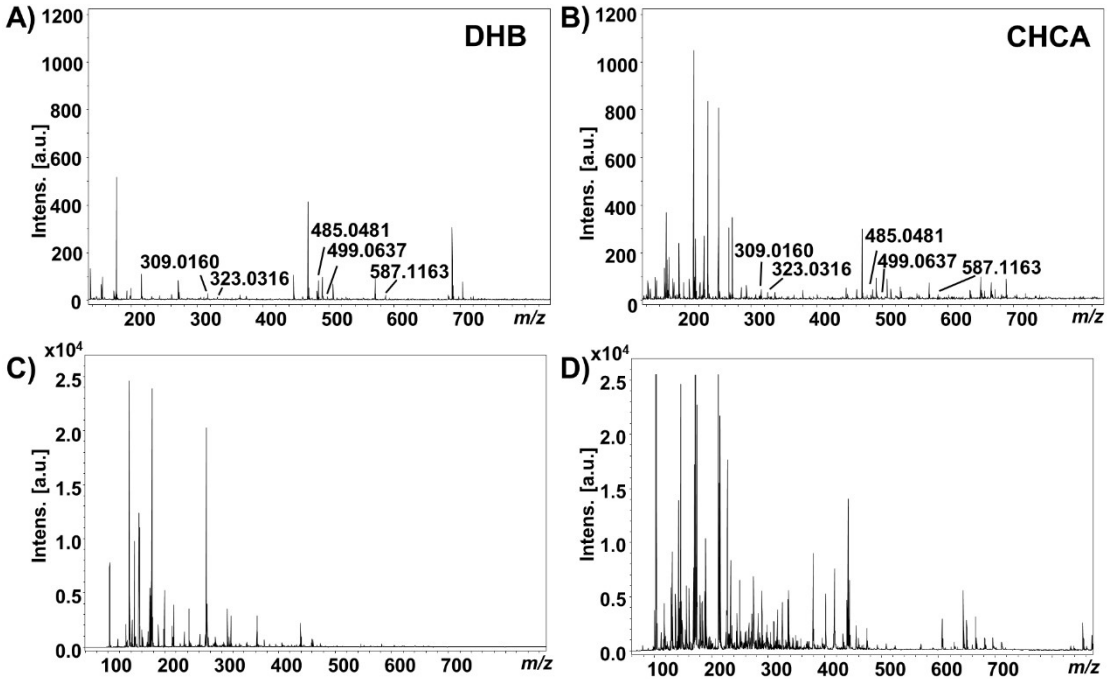

Figure S4. MALDI mass spectra acquired from root sections of *S. baicalensis* using A) DHB and B) CHCA in the positive ion mode, respectively. Background MALDI mass spectra obtained by using C) DHB and D) CHCA in the positive ion mode, respectively.

Table S1. Metabolite putatively assigned in *S. baicalensis* root tissue by positive ion MALDI MS using 3,4-DHB-TiO_2 CPs.

Classification	Compound	Calculated (m/z)	Measured (m/z)	Error (ppm)	Adduct ion	Molecular Formula
Free flavonoids						
	Baicalein	309.0160	309.0160	0.05	$[M + K]^+$	$C_{15}H_{10}O_5$
	Wogonin	323.0316	323.0311	-1.65	$[M + K]^+$	$C_{16}H_{12}O_5$
	Scutellarein	325.0109	325.0104	-1.53	$[M + K]^+$	$C_{15}H_{10}O_6$
	Trihydroxy-methoxyflavone	339.0265	339.0263	-0.73	$[M + K]^+$	$C_{16}H_{12}O_6$
	Dihydroxy-dimethoxyflavone	353.0422	353.0423	0.29	$[M + K]^+$	$C_{17}H_{14}O_6$
	Trihydroxy-dimethoxyflavone	369.0371	369.037	-0.31	$[M + K]^+$	$C_{17}H_{14}O_7$
	Dihydroxy-trimethoxyflavone	383.0528	383.0524	-0.95	$[M + K]^+$	$C_{18}H_{16}O_7$
	Viscidulin III	385.032	385.0321	0.19	$[M + K]^+$	$C_{17}H_{14}O_8$
	Skullcap flavone II	413.0633	413.0632	-0.31	$[M + K]^+$	$C_{19}H_{18}O_8$
Flavonoid glycosides						
	Chrysin-5-*O*-glucoside	455.0739	455.0741	0.45	$[M + K]^+$	$C_{21}H_{20}O_9$
	Chrysin-7-O-glucoside					
	Chrysin-7-*O*-glucuronide	469.0532	469.0531	-0.12	$[M + K]^+$	$C_{21}H_{18}O_{10}$
	Baicalein-7-*O*-glucoside	471.0688	471.0688	-0.02	$[M + K]^+$	$C_{21}H_{20}O_{10}$
	Norwogonin-7-O-glucoside					
	Dihydrobaicalein-7-*O*-glucoside	473.0845	473.0845	0.09	$[M + K]^+$	$C_{21}H_{22}O_{10}$
	Baicalin	485.0481	485.0481	0.06	$[M + K]^+$	$C_{21}H_{18}O_{11}$
	Wogonoside	499.0637	499.0636	-0.2	$[M + K]^+$	$C_{22}H_{20}O_{11}$
	Carthamidin-7-*O*-glucuronide	503.0586	503.0595	-0.27	$[M + K]^+$	$C_{21}H_{20}O_{12}$
	Isocarthamidin-7-*O*-glucuronide					
	Trihydroxy-methoxyflavone-glucuronide	515.0586	515.0584	-0.46	$[M + K]^+$	$C_{22}H_{20}O_{12}$
	Dihydroxy-dimethoxyflavone-glucuronide	529.0743	529.0743	0.02	$[M + K]^+$	$C_{23}H_{22}O_{12}$
	Trihydroxy-dimethoxyflavone-glucoside	531.0899	531.0898	-0.26	$[M + K]^+$	$C_{23}H_{24}O_{12}$
	Trihydroxy-dimethoxyflavone-glucuronide	545.0692	545.0694	0.36	$[M + K]^+$	$C_{23}H_{22}O_{13}$
	Chrysin-6-*C*-glucopyranoside-8-*C*-arabinopyranoside	587.1162	587.1163	0.25	$[M + K]^+$	$C_{26}H_{28}O_{13}$
	Chrysin-6-*C*-arabinopyranoside-8-*C*-glucopyranoside					
Other metabolites						
	Stigmasterol	451.3337	451.3336	-0.16	$[M + K]^+$	$C_{29}H_{48}O$
	Darendoside A	471.1263	471.126	-0.69	$[M + K]^+$	$C_{19}H_{28}O_{11}$
	Darendroside B	515.1525	515.1523	-0.39	$[M + K]^+$	$C_{21}H_{32}O_{12}$
	Daucosterol	615.4021	615.4022	0.08	$[M + K]^+$	$C_{35}H_{60}O_6$
	Martynoside	675.2259	675.2256	-0.51	$[M +Na]^+$	$C_{31}H_{40}O_{15}$

Table S2. Determination of major metabolites in *S. baicalensis* root tissues by LC-MS/MS.

No.	t_R/min	Molecular formula	Calculated (*m/z*)	Measured (*m/z*)	Error (ppm)	Compound	Structurally specific CID ions (*m/z*)
1	4 .013	$C_{21}H_{20}O_{12}$	463.0882	463.0855	-5.84	Carthamidin-7-O-glucuronide/Isocarthamidin-7-O-glucuronide	287.0531，166.9903
2	4.637	$C_{26}H_{28}O_{13}$	547.1457	547.1443	-2.59	Chrysin-6-C-glucopyranoside-8-C-arabinopyranoside/Chrysin-6-C-arabinopyranoside-8-C-glucopyranoside	529.1283，409.0874，391.0778
3	5.938	$C_{23}H_{24}O_{12}$	491.1195	491.1166	-5.91	Trihydroxy-dimethoxyflavone-glucoside	329.0599，314.0368，299.0134
4	6.008	$C_{21}H_{20}O_9$	415.1035	415.1014	-4.96	Chrysin-5-O-glucoside/Chrysin-7-O-glucoside	253.0392
5	6.013	$C_{23}H_{22}O_{13}$	505.0988	505.0972	-3.11	Trihydroxy-dimethoxyflavone-glucuronid	330.0289
6	6.975	$C_{21}H_{18}O_{11}$	445.0776	445.0763	-3.01	Baicalin	269.0416，251.0342，223.0323
7	7.643	$C_{15}H_{10}O_6$	285.0405	285.04	-1.63	Scutellarein	267.0262，193.0039
8	7.837	$C_{17}H_{14}O_8$	345.0616	345.0609	-2.01	Viscidulin III	330.0320，315.0077，287.0095，269.0028
9	8.111	$C_{21}H_{18}O_{10}$	429.0827	429.0812	-3.55	Chrysin-7-O-glucuronide	253.0465
10	8.156	$C_{22}H_{20}O_{12}$	475.0882	475.0871	-2.32	Trihydroxy-methoxyflavone-glucuronide	299.0473，284.0291
11	8.519	$C_{22}H_{20}O_{11}$	459.0933	459.0925	-1.72	Wogonoside	283.0567，268.0330
12	8.587	$C_{23}H_{22}O_{12}$	489.1039	489.0948	—	Dihydroxy-dimethoxyflavone-glucuronide	313.0671，298.0435
13	10.714	$C_{16}H_{12}O_6$	299.0561	299.0552	-3.05	Trihydroxy-methoxyflavone	284.0269，256.0287
14	11.135	$C_{15}H_{10}O_5$	269.0455	269.0451	-1.67	Baicalein	251.0282，223.0315
15	12.121	$C_{17}H_{14}O_7$	329.0667	329.0652	-4.49	Trihydroxy-dimethoxyflavone	314.0365，299.0133，271.0208
16	12.781	$C_{18}H_{16}O_7$	343.0823	343.0811	-3.58	Dihydroxy-trimethoxyflavone	328.0753，313.0283
17	13.022	$C_{16}H_{12}O_5$	283.0612	283.0607	-1.76	Wogonin	268.0327，239.0337
18	13.102	$C_{19}H_{18}O_8$	373.0929	373.0915	-3.74	Skullcap flavone II	358.0609，343.0388，325.0291
19	13.291	$C_{17}H_{14}O_6$	313.0718	313.0708	-3.08	Dihydroxy-dimethoxyflavone	283.0182，255.0176