Supporting Information (SI)

A novel binary matrix consisting of graphene oxide and caffeic acid for analysis of scutellarin and its metabolites in mouse kidney by MALDI Imaging

Tao Wang, [†a] Hin Kiu Lee, [†a] Grace Gar Lee Yue, [§] Arthur Chi Kong Chung, [†§*] Clara Bik San Lau, [§] Zongwei Cai [†*]

†. State Key Laboratory of Environmental and Biological Analysis, Department of Chemistry, Hong Kong Baptist University, Hong Kong, China;

§. Medicine & Therapeutics and Institute of Chinese Medicine and State Key Laboratory of Research on Bioactivities and Clinical Applications of Medicinal Plants, The Chinese University of Hong Kong, Hong Kong, China

***Corresponding author:** Arthur C.K. Chung, Zongwei Cai

Email: chungack@hkbu.edu.hk (Arthur C.K. Chung); zwcai@hkbu.edu.hk (Zongwei Cai)

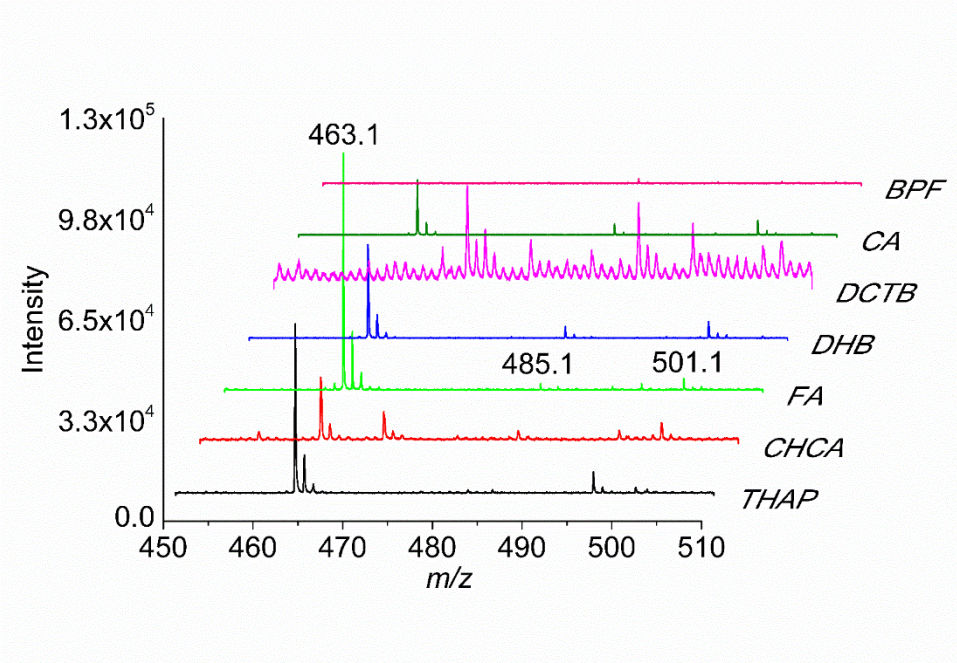

Figure S1. MALDI-TOF MS analysis of scutellarin standard with different matrices were tested on blank tissue sections in positive ionization mode. 1 μL of the matrix was pre-mixed with 1 μL scutellarin standard (0.05 mg/ml). The solutions were then pipetted onto the blank tissue section, and were dried under vacuum until analysis by MALDI-MS. All measurements were conducted in the positive ion mode. Scutellarin was detected as the molecular ion, sodium and potassium adduct ion, $[M+H]^+$ at *m/z* 463.1, $[M+Na]^+$ at *m/z* 485.1 and $[M+K]^+$ ion at *m/z* 501.1.

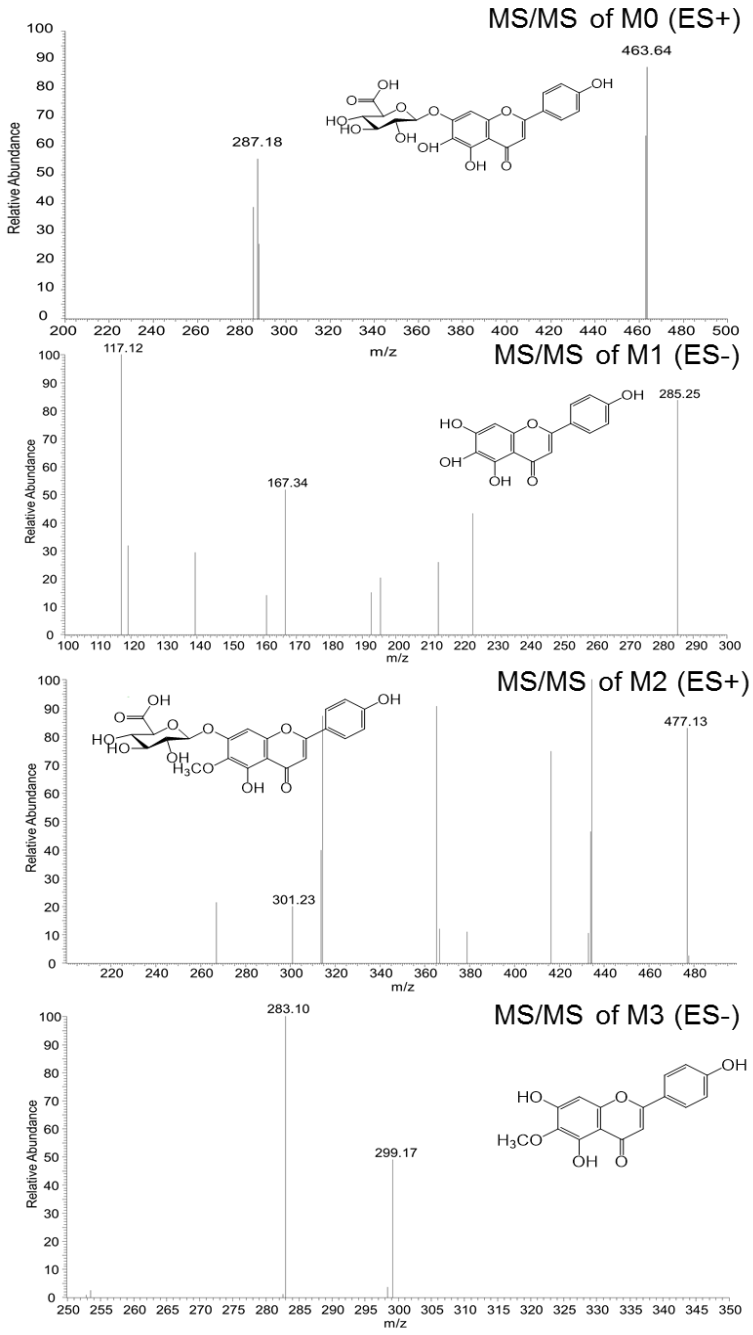

Figure S2. MS/MS spectra of scutellarin and its metabolites (M0-M3) in both positive and negative ion mode.[1-3]

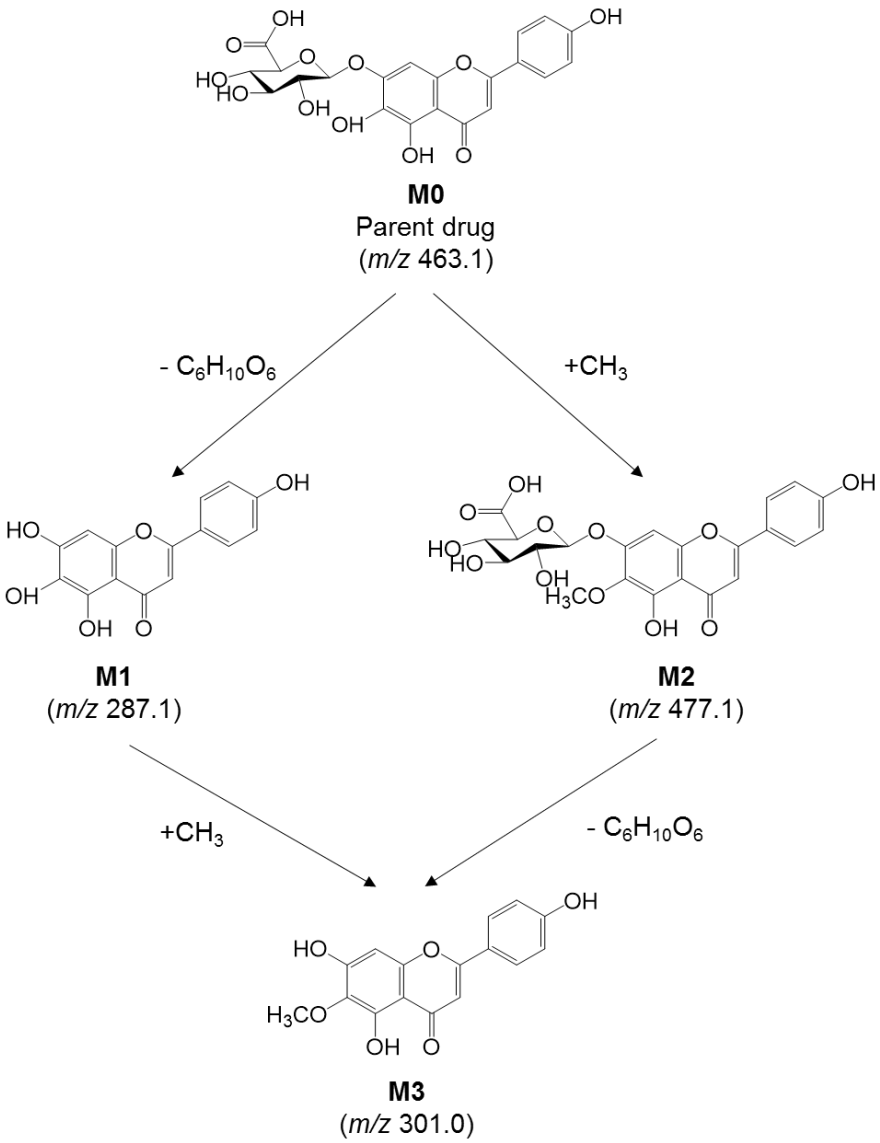

Figure S3. Proposed biotransformation pathways and structures of scutellarin and its metabolites.[4]

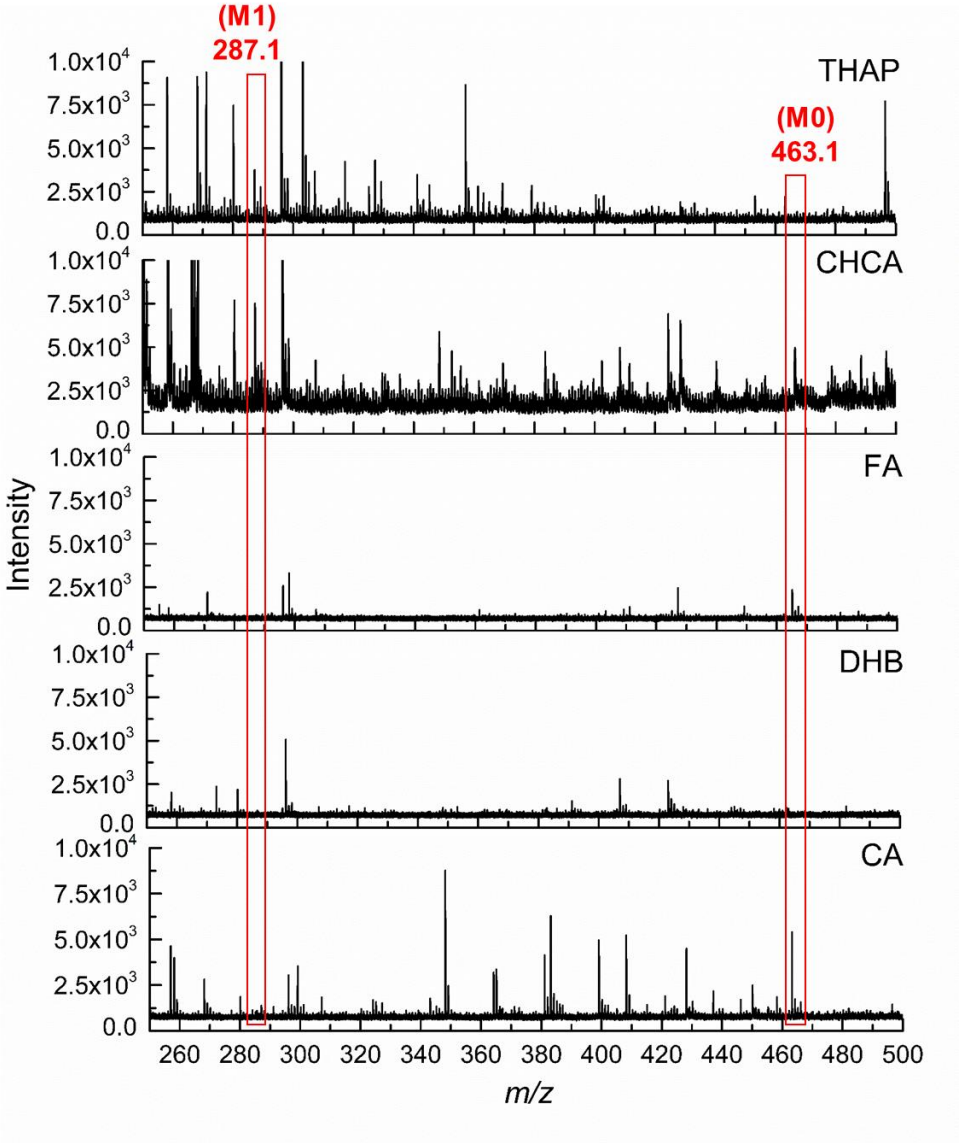

Figure S4. The MALDI mass spectra of scutellarin (*m/z* 463.1) and its metabolites (*m/z* 287.1, *m/z* 477.1, *m/z* 301.0) with THAP, CHCA, FA, DHB and CA matrix in the positive ion mode. The matrix was deposited on scutellarin-treated kidney tissue sections.

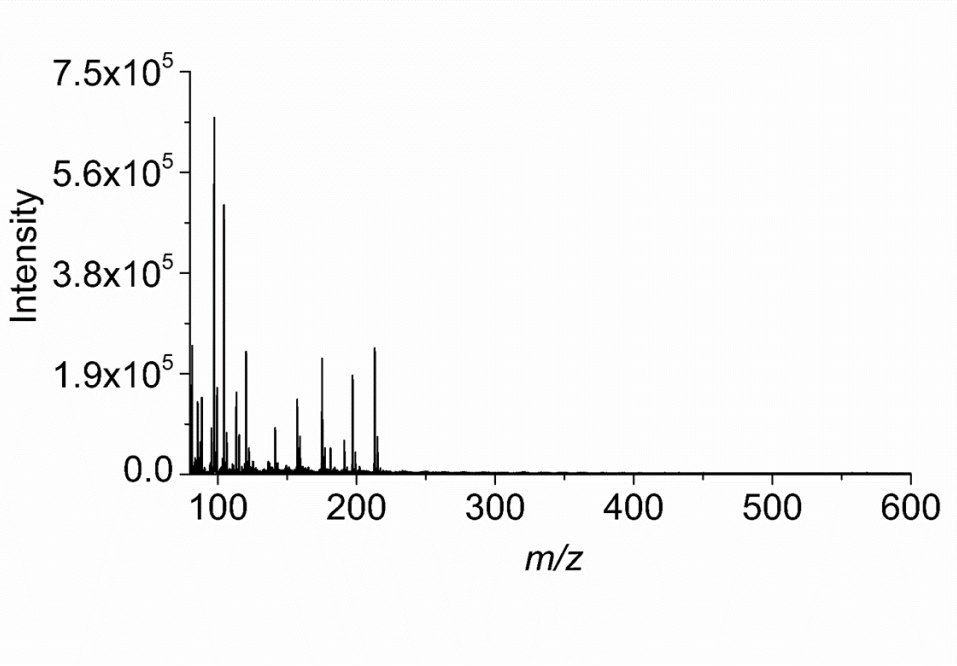

Figure S5. The MALDI mass spectrum of scutellarin and its metabolites with GO as matrix in the positive ion mode.

Figure S6. Optical microscope image on the coverage of GO/CA on three different tissue sections.

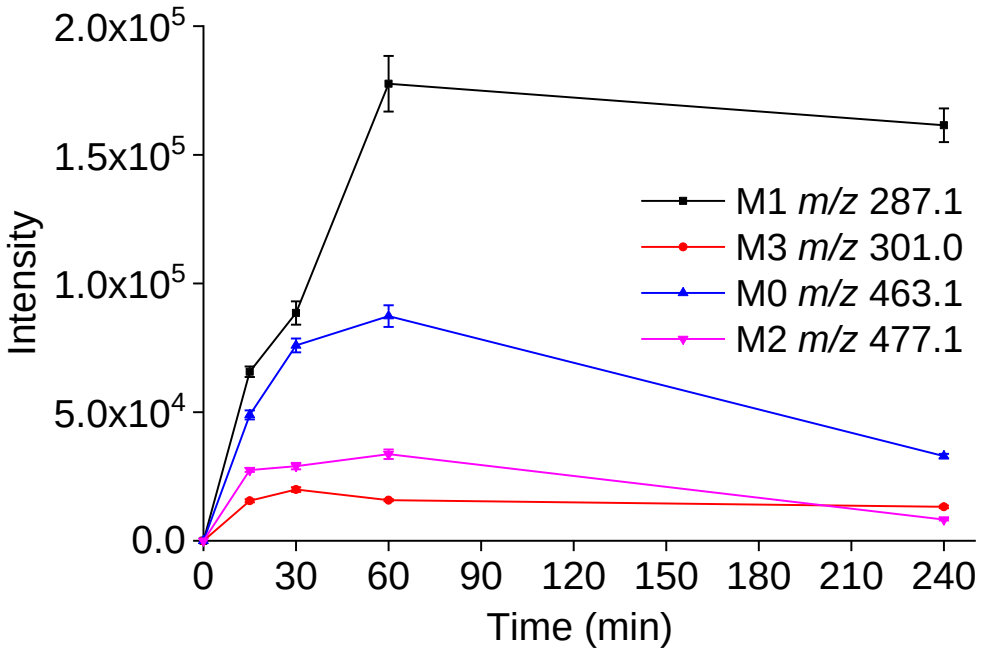

Figure S7. Time profiles of scutellarin and its metabolites determined in kidney after ip injection by MALDI-MS.

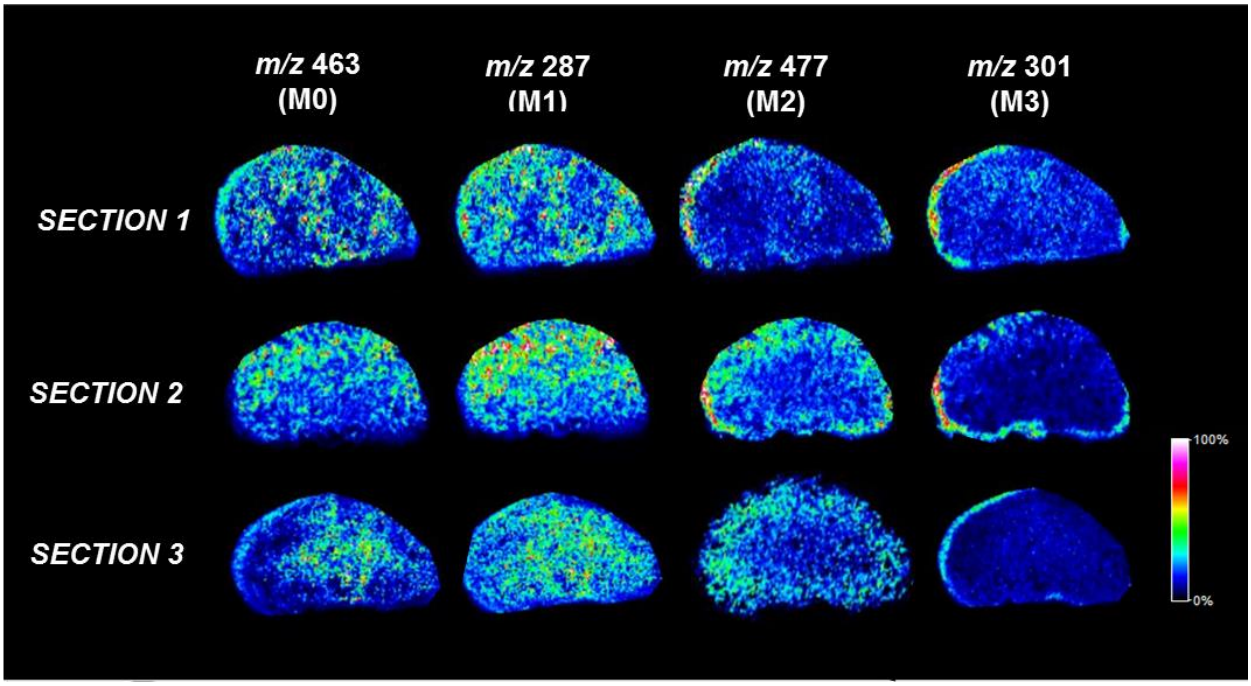

Figure S8. Reproducibility imaging analysis of scutellarin and its metabolites by MALDI-MSI.

Table S1. Preparation of Matrix Solutions

Matrix	Concentration	Solvent mixture
Ferulic acid (FA)	15 mg/ml	MeOH / 0.1%TFA
Caffeic acid (CA)	15 mg/ml	MeOH / 0.1%TFA
2,5-Dihydroxybenzoic acid (DHB)	15 mg/ml	ACN:H_2O (1:1) / 0.1%TFA
a-Cyano-4-hydroxycinnamic acid (CHCA)	15 mg/ml	MeOH / 0.1%TFA
2,4,6-Trihydroxyacetophenone (THAP)	15 mg/ml	ACN:H_2O (1:1) / 0.1%TFA
trans-2-[3-(4-tert-Butylphenyl)-2-methyl-2-propenylidene]malononitrile (DCTB)	15 mg/ml	DCM / 0.1%TFA
bis(4-hydroxyphenyl)methane (BPF)	15 mg/ml	MeOH / 0.1%TFA
Graphene oxide (GO)	8 mg/ml	MeOH/H_2O (1:1)

Table S2. Before and after comparison analysis of adding matrix GO by MALDI-MS

Matrix	Ion	m/z	S/N ratio	
			Matrix	Matrix+ GO
CA	M0	463.1	25.72	54.05
	M1	287.1	≤ 3	19.28
	M2	477.1	≤ 3	8.44
	M3	301.0	/	/
CHCA	M0	463.1	≤ 3	7.84
	M1	287.1	10.86	51.76
	M2	477.1	≤ 3	6.65
	M3	301.0	/	/
THAP	M0	463.1	6.82	/
	M1	287.1	7.19	≤ 3
	M2	477.1	/	/
	M3	301.0	/	/

Reference

1. J. L. Zhang, Q. M. Che, S. Z. Li and T. H. Zhou, *J Asian Nat. Prod. Res.*, 2003, **5**, 249-256.

2. H. Tang, N. G. Li, Y. P. Tang, Q. P. Shi, J. M. Guo, W. Zhang, M. Z. Shen and J. A. Duan, *Anal. Methods*, 2014, **6**, 4667-4673.

3. X. Chen, L. Cui, X. Duan, B. Ma and D. Zhong, *Drug metab. Dispos.*, 2006, **34**, 1345-1352.

4. L. P. Wang and Q. Ma, *Pharmacol. Ther.*, 2018, **190**, 105-127.