Electronic Supplementary Information

On the use of a 2D-carbon microfiber fractionation system to improve flow-injection QTOF-HRMS analysis in complex matrices: the case of *Abelmoschus manihot* flower extracts

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Part1 **Fig. S1-S6**: 2DμCFs-QTOF-MS/MS spectra of the six predicted compounds. (S3-S5)

Part2 **Fig. S7-S13**: Extracted ion chromatograms (EIC) of the seven co-elution compounds by HPLC-MS. (S6-S8)

Part3 **Table S1**: List of investigated compounds obtained by the 2DμCFs-QTOF-MS/MS from *A. manihot* flower extracts. (S9-S10)

Part4 **Table S2**: Putative identification of chemical constituents of *A. manihot* flower extracts by using HPLC-MS/MS in positive ion mode. (S11-S12)

Part5 **References**: S13- S14

Part1: 2DμCFs-QTOF-MS/MS spectra of the six predicted compounds. （Fig. S1-S6）

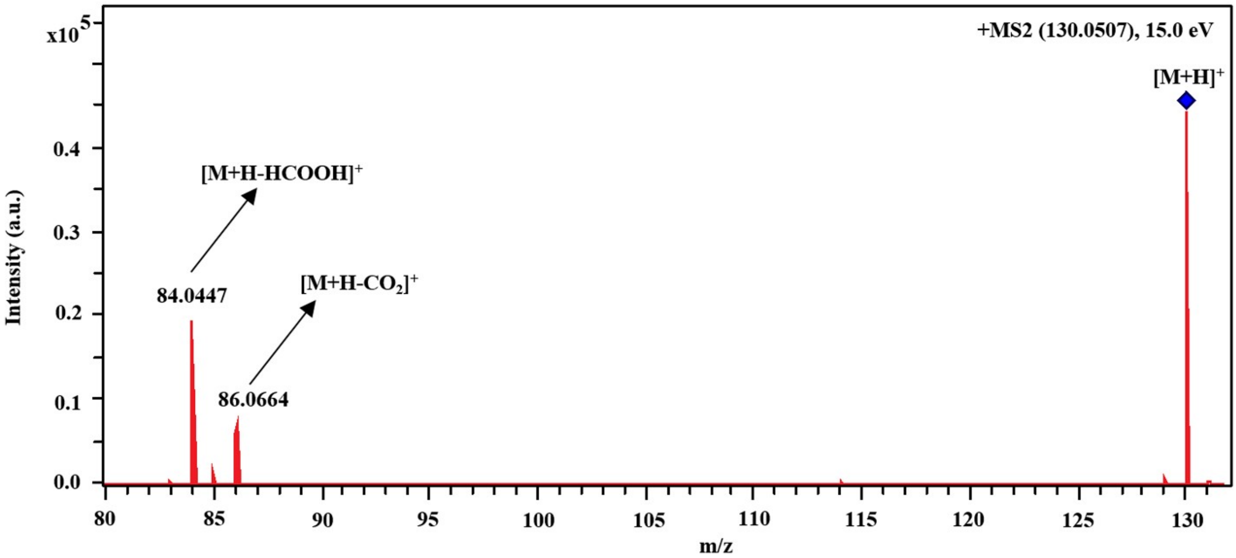

Fig. S1 Fragmentation results for m/z 130.0507 by 2DμCFs-QTOF-MS/MS in positive ion mode.

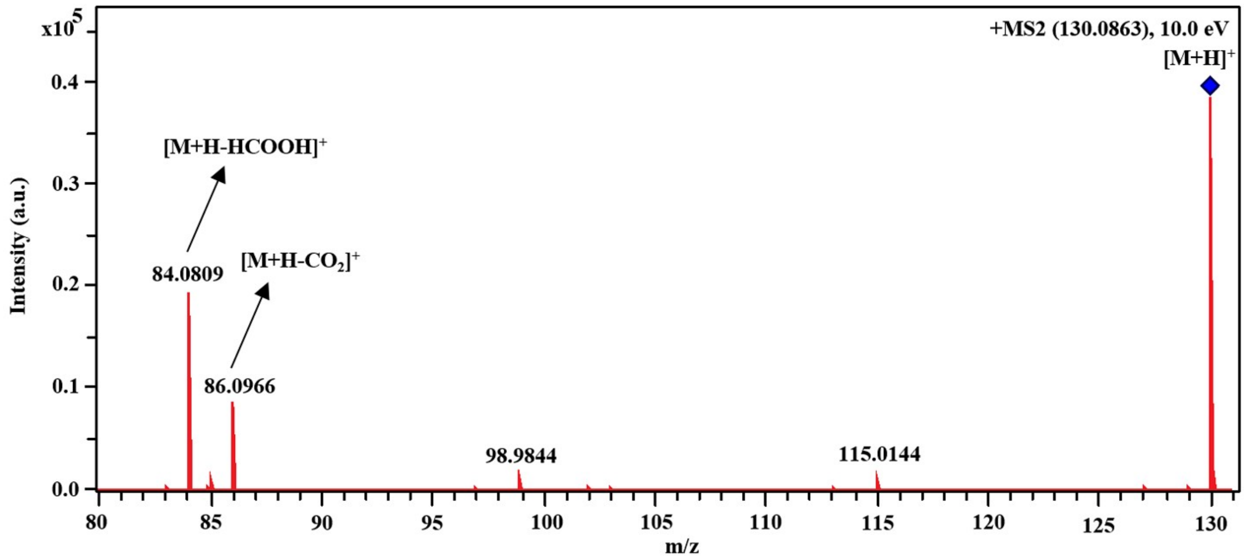

Fig. S2 Fragmentation results for m/z 130.0863 by 2DμCFs-QTOF-MS/MS in positive ion mode.

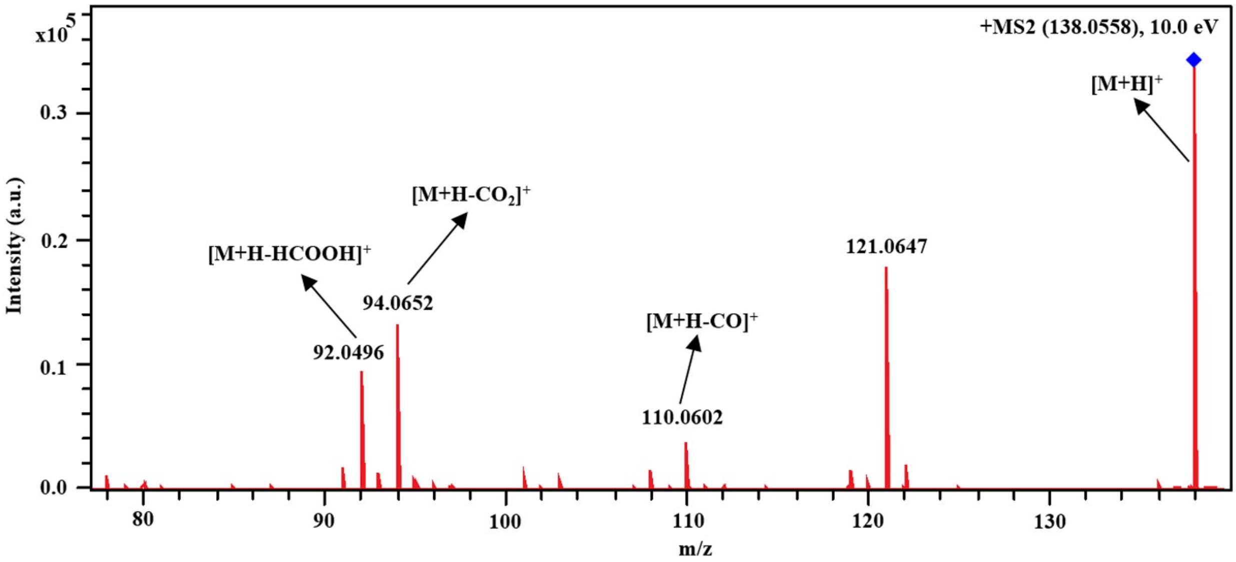

Fig. S3 Fragmentation results for m/z 138.0558 by 2DμCFs-QTOF-MS/MS in positive ion mode.

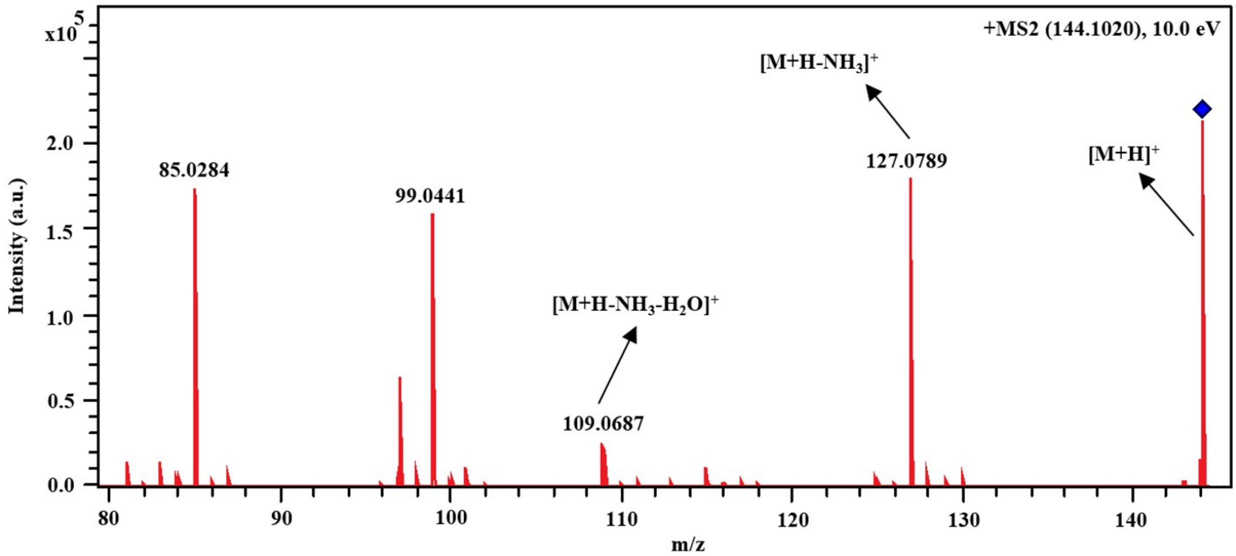

Fig. S4 Fragmentation results for m/z 144.1020 by 2DμCFs-QTOF-MS/MS in positive ion mode.

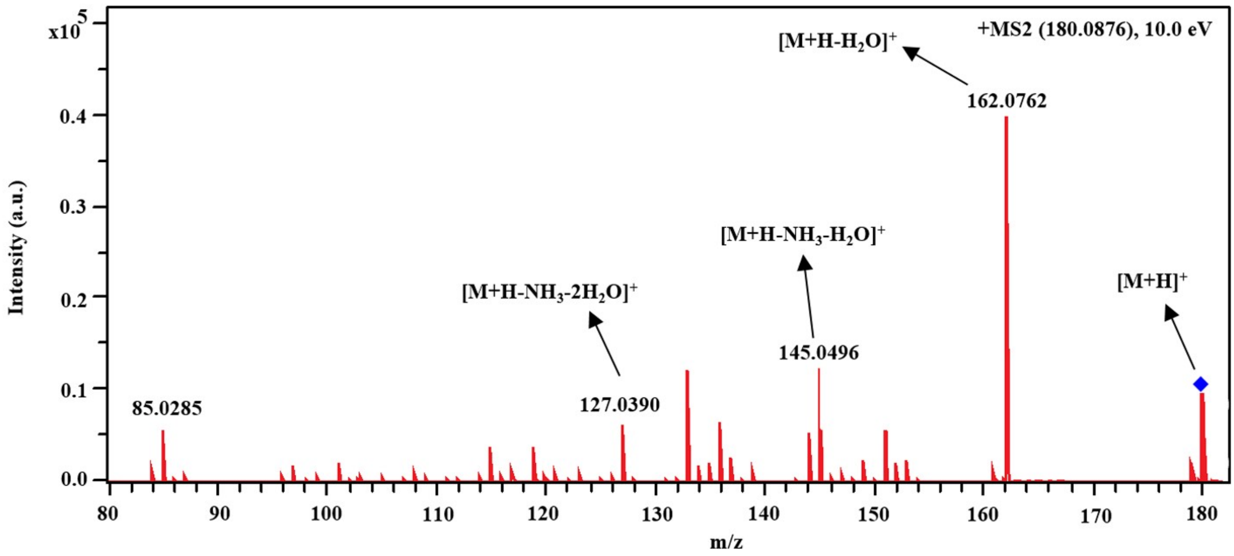

Fig. S5 Fragmentation results for m/z 180.0876 by 2DμCFs-QTOF-MS/MS in positive ion mode.

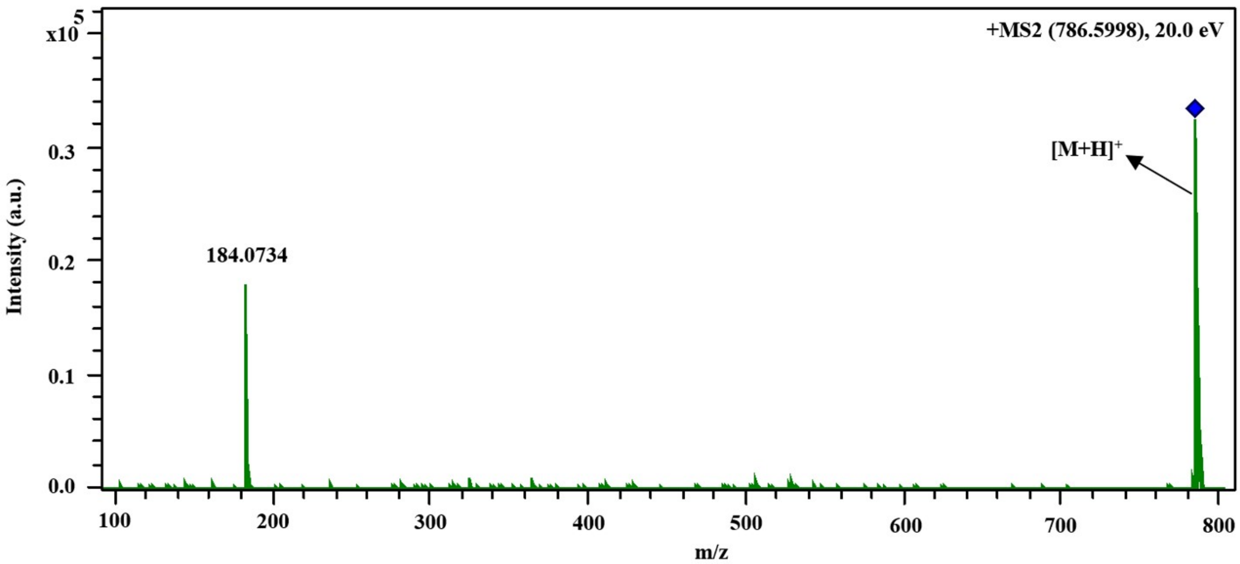

Fig. S6 Fragmentation results for m/z 786.5998 by 2DμCFs-QTOF-MS/MS in positive ion mode.

Part2：Extracted ion chromatograms (EIC) of the seven co-elution compounds. （Fig. S7-S13）

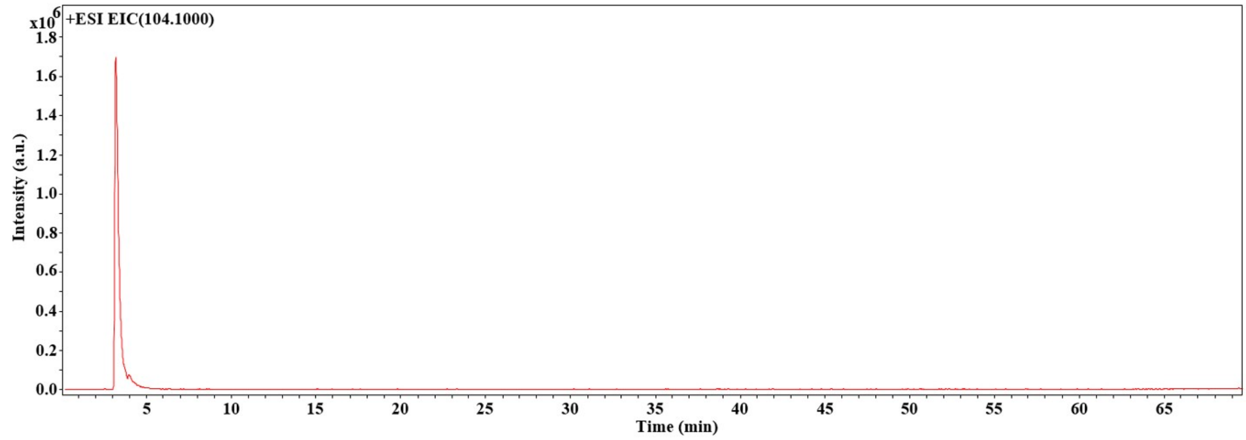

Fig. S7 Extraction of ion chromatogram (EIC) of m/z 104.1 by HPLC-MS in positive ion mode.

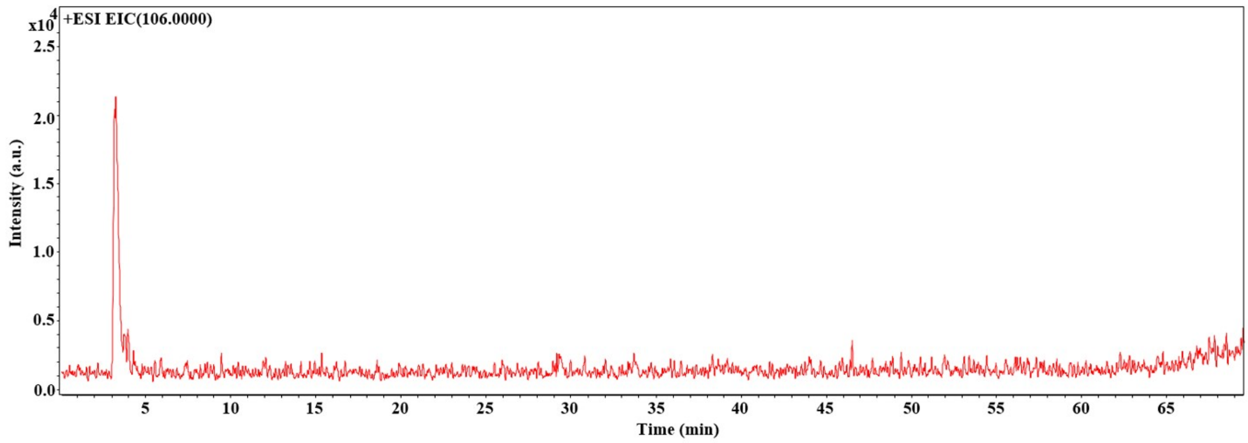

Fig. S8 Extraction of ion chromatogram (EIC) of m/z 106.0 by HPLC-MS in positive ion mode.

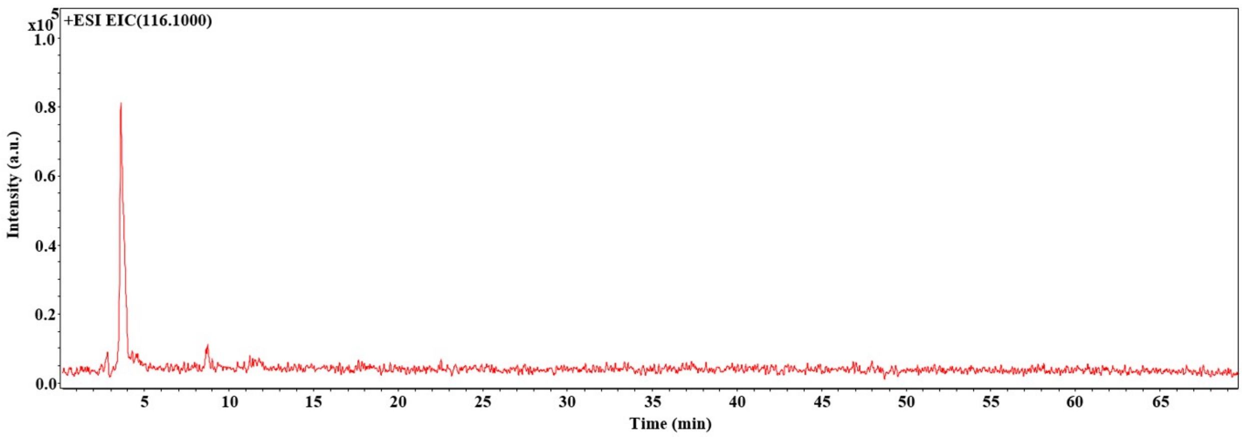

Fig. S9 Extraction of ion chromatogram (EIC) of m/z 116.1 by HPLC-MS in positive ion mode.

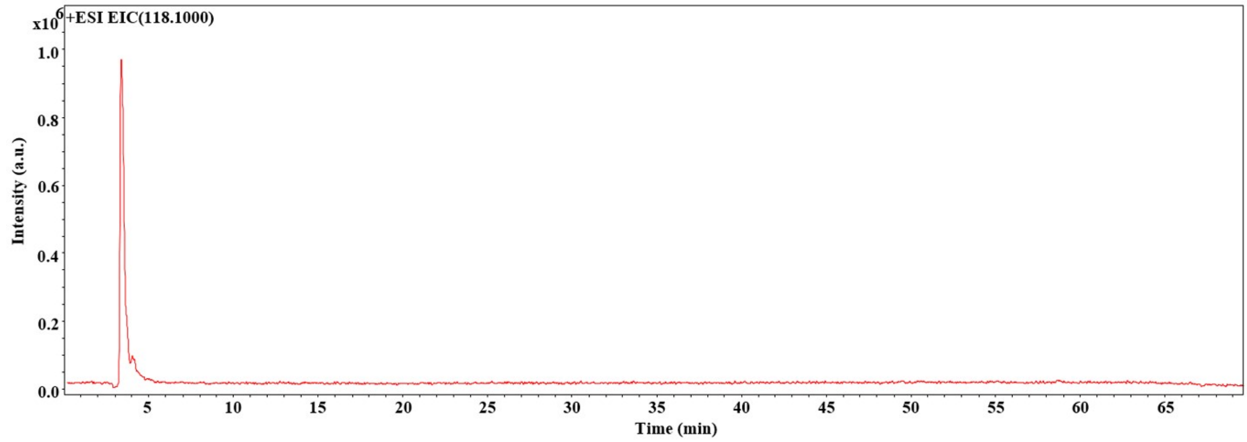

Fig. S10 Extraction of ion chromatogram (EIC) of m/z 118.1 by HPLC-MS in positive ion mode.

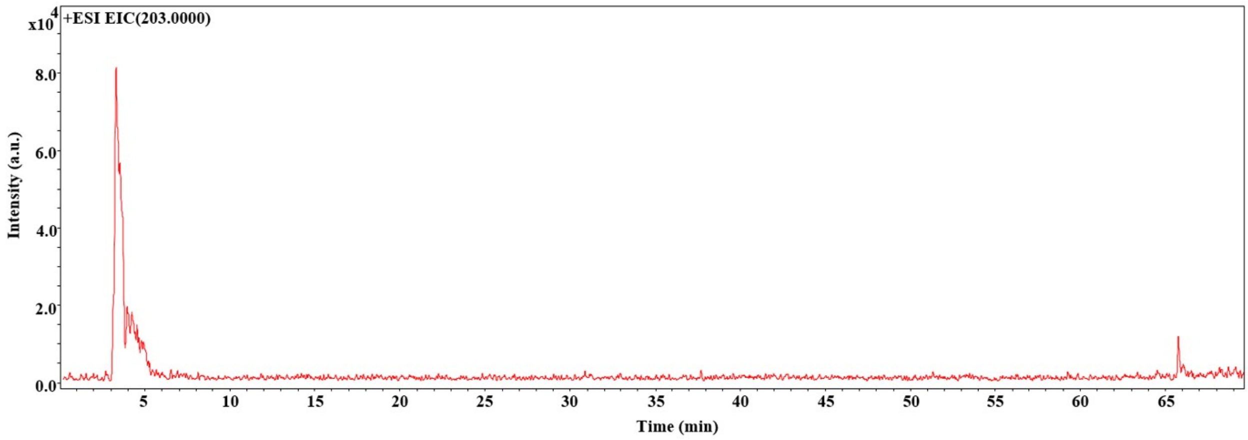

Fig. S11 Extraction of ion chromatogram (EIC) of m/z 203.0 by HPLC-MS in positive ion mode.

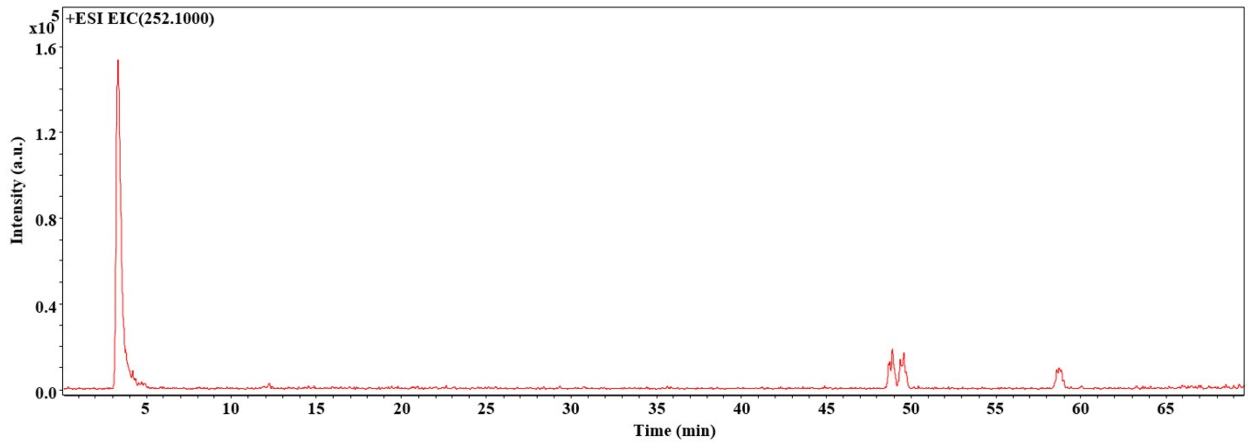

Fig. S12 Extraction of ion chromatogram (EIC) of m/z 252.1 by HPLC-MS in positive ion mode.

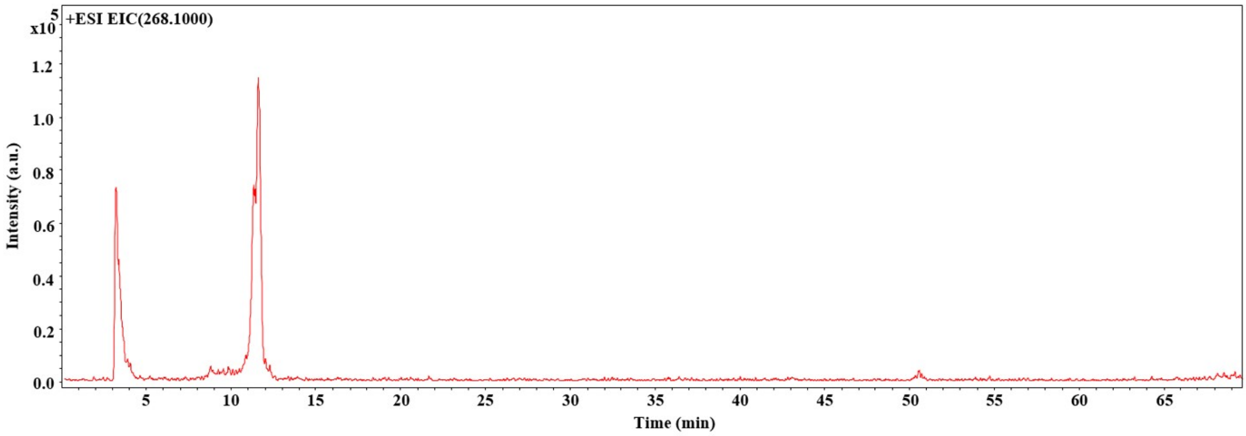

Fig. S13 Extraction of ion chromatogram (EIC) of m/z 268.1 by HPLC-MS in positive ion mode.

Part3：

Table S1 List of investigated compounds obtained by the 2DμCFs-QTOF-MS/MS from *A. manihot* flower extracts.

Peak NO.	Fraction	Compound	Formula	Adduct	Calculated mass (Da)	Observed mass (Da)	Score	Err [ppm]	Fragment ions (m/z)	Ref
1	High-polar	γ-Aminobutyric acid	$C_4H_9NO_2$	$[M+H]^+$	104.0706	104.0714	100	-7.7	87.0444； 86.0604	1
2		Serine	$C_3H_7NO_3$	$[M+H]^+$	106.0499	106.0506	100	-6.6	88.0398	1
3		Proline	$C_5H_9NO_2$	$[M+H]^+$	116.0706	116.0714	100	-6.8	70.0663	1
4		Valine	$C_5H_{11}NO_2$	$[M+H]^+$	118.0863	118.0871	100	-6.7	72.0809	1
7		Isoleucine/Leucine*	$C_6H_{13}NO_2$	$[M+H]^+$	132.1019	132.1027	100	-6.0	86.0965	1
8		Aspartic acid	$C_4H_7NO_4$	$[M+H]^+$	134.0448	134.0459	100	-8.2	88.0394； 116.0341	1
9		Adenine	$C_5H_5N_5$	$[M+H]^+$	136.0627	136.0633	100	-4.4	119.0346	2
11		5-(hydroxymethyl)-2-furancarboxylic acid	$C_6H_6O_4$	$[M+H]^+$	143.0339	143.0345	100	-4.2		3
13		Glutamine	$C_5H_{10}N_2O_3$	$[M+H]^+$	147.0764	147.0773	100	-6.1	84.0445	4
14		Lysine	$C_6H_{14}N_2O_2$	$[M+H]^+$	147.1128	147.1131	100	-2.0	84.0820； 130.0875	1
15		Glutamate	$C_5H_9NO_4$	$[M+H]^+$	148.0604	148.0613	100	-6.0	84.0446； 130.0501； 102.0548	1
16		Guanine	$C_5H_5N_5O$	$[M+H]^+$	152.0566	152.0572	100	-3.9	93.0092	2
17		Phenylalanine	$C_9H_{11}NO_2$	$[M+H]^+$	166.0863	166.0871	100	-4.8	120.0808	3
19		Tyrosine	$C_9H_{11}NO_3$	$[M+H]^+$	182.0812	182.0820	100	-4.3	136.0762； 123.0445	1
20		Galactose/Glucose/Mannose*	$C_6H_{12}O_6$	$[M+Na]^+$	203.0526	203.0536	100	-4.9		5
21		Tryptophan	$C_{11}H_{12}N_2O_2$	$[M+H]^+$	205.0972	205.0986	100	-6.8	146.0610； 188.0715	1
22		Unknown H1	$C_{11}H_6O_5$	$[M+H]^+$	219.0288	219.0275	100	5.9	202.0710； 197.0286	
23		Cytidine	$C_9H_{13}N_3O_5$	$[M+H]^+$	244.0928	244.0927	100	0.4	112.0508	2
24		2'-Deoxyadenosine	$C_{10}H_{13}N_5O_3$	$[M+H]^+$	252.1091	252.1088	100	1.1	99.0443； 136.0624； 117.0545	2
25		Adenosine/2'-Deoxyguanosine*	$C_{10}H_{13}N_5O_4$	$[M+H]^+$	268.1040	268.1047	100	-2.6	136.0621； 117.0548	3
26		Unknown H2	$C_{12}H_{16}N_6O_4$	$[M+H]^+$	309.1306	309.1303	100	0.9	291.1189； 273.1083； 225.0872； 292.1030； 292.1217； 130.0862	
27		Unknown H3	$C_{17}H_{16}O_{10}$	$[M+H]^+$	381.0816	381.0804	100	3.1	203.0527； 364.1238； 290.0762；	

									219.0266；201.0162；274.0924	
28	Medium-polar	Uridine	$C_9H_{12}N_2O_6$	$[M+H]^+$	245.0768	245.0780	100	-4.8	113.0346	6
29		Quercetin	$C_{15}H_{10}O_7$	$[M+H]^+$	303.0499	303.0513	100	-4.6	274.0437	7
30		Myricetin	$C_{15}H_{10}O_8$	$[M+H]^+$	319.0448	319.0460	100	-3.7	153.0178；273.0397	8
31		Chlorogenic Acid	$C_{16}H_{18}O_9$	$[M+H]^+$	355.1024	355.1037	100	-3.6	163.0395	3
32		Quercetin 7-O-glucoside/Hyperin/ Isoquercetin/Quercetin 3'-O-glucoside*	$C_{21}H_{20}O_{12}$	$[M+H]^+$	465.1028	465.1043	100	-3.2	303.0506；85.0288；91.0395；97.0289；127.0396	7
33		Myricetin 3-O-glucoside/ Myricetin 3'-O-glucoside*	$C_{21}H_{20}O_{13}$	$[M+H]^+$	481.0977	481.0989	100	-2.4		3
34		Unknown M1	$C_{20}H_{10}N_{10}O_6$	$[M+H]^+$	487.0858	487.0860	100	-0.4		
35		Quercetin 3-O-(6-acetylglucoside)	$C_{23}H_{22}O_{13}$	$[M+H]^+$	507.1133	507.1143	100	-1.9		3
36		Quercetin 3-O-robinobioside/Rutin*	$C_{27}H_{30}O_{16}$	$[M+H]^+$	611.1607	611.1632	100	-4.0	303.0506；465.1040；129.0552；147.0679；85.0288	8
37		Unknown M2	$C_{44}H_{38}O_{24}$	$[M+H]^+$	951.1826	951.1810	100	1.6		
38	Weak-polar	Linoleic acid	$C_{18}H_{32}O_2$	$[M+H]^+$	281.2475	281.2480	100	-1.7	263.2368；245.2266；83.0858；97.1012；111.1158	4
39		Oleic Acid	$C_{18}H_{34}O_2$	$[M+H]^+$	283.2632	283.2638	100	-2.1	265.2524；247.2418；135.1176	4
40		Unknown W1	$C_{29}H_{48}O_2$	$[M+H]^+$	429.3727	429.3735	100	-1.8	383.1036；249.0393；219.0265；293.0720；323.0828；250.0470	
41		Tocopherol	$C_{29}H_{50}O_2$	$[M+H]^+$	431.3884	431.3872	100	2.7	165.0919	9
42		Hibifolin	$C_{21}H_{18}O_{14}$	$[M+H]^+$	495.0769	495.0785	100	-3.2		7
43		Unknown W2	$C_{33}H_{45}NO_4$	$[M+H]^+$	520.3421	520.3424	100	-0.5	130.0864；180.0870；104.0706；252.1077；268.1034；184.0731	
44		Unknown W3	$C_{29}H_{43}N_7O_2$	$[M+H]^+$	522.3551	522.3556	100	-0.9	180.0868；104.0707；184.0736；325.1127；391.2841；162.0765	
45		Lutein	$C_{40}H_{56}O_2$	$[M+H]^+$	569.4353	569.4364	100	-1.9	476.3653；175.1493	10
46		Unknown W4	$C_{35}H_{36}N_4O_5$	$[M+H]^+$	593.2758	593.2773	100	-2.5	252.1081；253.1114；234.0970；465.1023；415.0985；266.1238	

47	Unknown W5	$C_{44}H_{77}N_3O_7$	$[M+H]^+$	760.5816	760.5853	100	-4.8	104.0707；130.0867；184.0734；294.1550；418.1551；420.0900
49	Unknown W6	$C_{55}H_{74}N_4O_5$	$[M+H]^+$	871.5732	871.5731	100	0.1	

*: Isobaric compounds

Part4：

Table S2 Putative identification of chemical constituents of *A. manihot* flower extracts by HPLC-MS/MS in positive ion mode.

Peak NO.	Analyte	t_R(min)	Formula	$[M+H]^+$(m/z)	Fragment ions (m/z)	Ref
1	5-(hydroxymethyl)-2-furancarboxylic acid	2.70	$C_6H_6O_4$	143.0	99.0	3
2	Serine	3.20	$C_3H_7NO_3$	106.0	88.0,60	1,3
3	γ-Aminobutyric acid	3.22	$C_4H_9NO_2$	104.1	86.0,87.0	1
4	Adenosine	3.32	$C_{10}H_{13}N_5O_4$	268.1	136.1	3
5	2'-Deoxyadenosine	3.32	$C_{10}H_{13}N_5O_3$	252.1	99.0,136.1,117.1	2
6	Glucose	3.37	$C_6H_{12}O_6$	203.0		11
7	Valine	3.42	$C_5H_{11}NO_2$	118.1	72.1	1,3
8	Proline	3.61	$C_5H_9NO_2$	116.1	70.0	1,3
9	Unknown 1	3.87		260.0		
10	Phenylalanine	4.01	$C_9H_{11}NO_2$	166.1	120.1	1,3
11	Cytidine	4.08	$C_9H_{13}N_3O_5$	244.1	112.1	2
12	Unknown 2	5.30		130.0		
13	Unknown 3	5.78		348.0		
14	Unknown 4	6.42		238.1		
15	Leucine	9.06	$C_6H_{13}NO_2$	132.1	86.1	1,3
16	Unknown 5	15.28		120.0		
17	Unknown 6	25.44		298.0		
18	Unknown 7	38.01		371.1		
19	Unknown 8	39.15		657.0		
20	Unknown 9	40.00		479.0		
21	Unknown 10	41.98		476.1		
22	Unknown 11	43.42		402.0		
23	Unknown 12	43.96		520.1		
24	Myricetin 3-O-glucoside	44.47	$C_{21}H_{20}O_{13}$	481.1	318.0	3
25	Lutein	45.80	$C_{40}H_{56}O_2$	569.4	175.1,476.3	10
26	Quercetin 3-O-robinobioside	47.23	$C_{27}H_{30}O_{16}$	611.0	303.0,465.0,129.1	3
27	Quercetin 7-O-glucoside	47.24	$C_{21}H_{20}O_{12}$	465.0	303.1,465.0	3
28	Rutin	47.63	$C_{27}H_{30}O_{16}$	611.0	85.0,129.1,147.1 303.1,465.1	3,7
29	Hyperin	48.85	$C_{21}H_{20}O_{12}$	465.0	303.0,273.1,257.0, 181.0,153.1	3,7
30	Isoquercetin	49.53	$C_{21}H_{20}O_{12}$	465.0	303.0,273.1,257.1 181.0,153.0	3,7
31	Myricetin 3'-O-glucoside	50.59	$C_{21}H_{20}O_{13}$	481.1	319.1	3
32	Unknown 13	52.28		551.0		
33	Hibifolin	54.79	$C_{21}H_{18}O_{14}$	495.1	319.0	3,7
34	Tocopherol	58.36	$C_{29}H_{50}O_2$	431.3	165.1	9
35	Myricetin	58.37	$C_{15}H_{10}O_8$	319.0	153.0,273.0,181.1	7
36	Quercetin 3'-O-glucoside	58.70	$C_{21}H_{20}O_{12}$	465.0	85.0,91.0,97.0,303.1, 127.0	3,7

37	Unknown 14	61.73		551.0		
38	Quercetin	64.84	$C_{15}H_{10}O_7$	303.0	274.0,153.1	7
39	Unknown 15	65.75		219.1		
40	Unknown 16	67.14		274.2		
41	Unknown 17	68.09		343.1		
42	Uridine	68.48	$C_9H_{12}N_2O_6$	245.1	113.0	6
43	Linoleic acid	69.09	$C_{18}H_{32}O_2$	281.2	83.1,97.1,111.1, 245.2,263.2	4
44	Oleic Acid	69.39	$C_{18}H_{34}O_2$	283.2	135.1,247.2,265.2	4

Part5：

References

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