Supporting information

Figure legends

Figure S1. Metabolomics analysis of scoparone metabolites in positive ionization mode. (A) OPLS-DA score plots analysis of feces between vehicle treatment mice and scoparone treatment mice. (B) OPLS-DA score plots analysis of urine between vehicle treatment mice and scoparone treatment mice. (C) S-plots observed in OPLS-DA analysis of feces between vehicle-treated group and scoparone-treated group. The potential metabolites were marked in the S-plots. (D) S-plots observed in OPLS-DA analysis of urine between vehicle-treated group and scoparone-treated group.

Figure S2. Metabolomics analysis of scoparone metabolites in negative ionization mode. (A) OPLS-DA score plots analysis of plasma between vehicle treatment mice and scoparone treatment mice. (B) S-plots observed in OPLS-DA analysis of plasma between vehicle-treated group and scoparone-treated group. The potential metabolites were marked in the S-plots. (C) OPLS-DA score plots analysis of MLM between vehicle treatment mice and scoparone treatment mice. (D) S-plots observed in OPLS-DA analysis of MLMs between vehicle-treated group and scoparone-treated group. (E) Trend plot of Msa4 in urine with and without scoparone (●, control group; ▲, scoparone group). (F) Trend plot of Msa17 in urine with and without scoparone (●, control group; ▲, scoparone group).

Figure S3. MMDF analysis of scoparone metabolites. (A) Mass defect plots of urine from scoparone-treated mice. This mass defect filter was performed using hydroxylated and glucoronate scoparone molecular as the filter template in negative ionization mode. The potential metabolite points were labeled in mass defect. (B) The chromatograms of scoparone metabolites in urine filtered by mass defect filter technique with hydroxylated and glucoronate scoparone molecular filter template in negative ionization mode. (C) Mass defect plots of urine from scoparone-treated mice. This mass defect filter was performed using hydroxylation and sulfate scoparone molecular as the filter template in negative ionization mode. The potential metabolite points were labeled in mass defect. (D) The chromatograms of scoparone metabolites in urine filtered by mass defect filter technique with hydroxylation and sulfate scoparone molecular filter template in negative ionization mode. (E) Mass defect plots of feces from scoparone-treated mice. This mass defect filter was performed using scoparone molecular as the filter template in positive ionization mode. The potential metabolite points were labeled in mass defect. (F) The chromatograms of scoparone metabolites in urine filtered by mass defect filter technique with scoparone molecular filter template in positive ionization mode.

Figure S4. Metabolomics analysis of scopoletin metabolites in urine. (A) OPLS-DA score plots analysis of urine between vehicle treatment mice and scopoletin treatment mice (in negative ionization mode). (B) S-plots observed in OPLS-DA analysis of urine between vehicle-treated group and esculetin-treated group. The potential metabolites were marked in the S-plots. (C) Trend plot of Mso6 in urine with and

without scopoletin (●, control group; ▲, scopoletin group). (D) Trend plot of Me2 in urine with and without esculetin (●, control group; ▲, esculetin group).

Figure S5. Metabolomics analysis of scopoletin metabolites in feces, plasma, and microsome. (A) OPLS-DA score plots analysis of feces between vehicle treatment mice and scopoletin treatment mice (in negative ionization mode). (B) S-plots observed in OPLS-DA analysis of feces between vehicle-treated group and scopoletin-treated group (in negative ionization mode). The potential metabolites were marked in the S-plots. (C) OPLS-DA score plots analysis of plasma between vehicle treatment mice and esculetin treatment mice (in negative ionization mode). (D) S-plots observed in OPLS-DA analysis of plasma between vehicle-treated group and esculetin-treated group (in negative ionization mode). The potential metabolites were marked in the S-plots. (E) OPLS-DA score plots analysis of MLMs between vehicle treatment mice and esculetin treatment mice (in negative ionization mode). (F) S-plots observed in OPLS-DA analysis of MLMs between vehicle-treated group and esculetin-treated group (in negative ionization mode). The potential metabolites were marked in the S-plots.

Figure S6. MMDF analysis of scopoletin metabolites. (A) Mass defect plots of urine from scopoletin-treated mice. This mass defect filter was performed using glucuronate scopoletin molecular as the filter template in negative ionization mode. The potential metabolite points were labeled in mass defect. (B) The chromatograms of scopoletin metabolites in urine filtered by mass defect filter technique with glucuronate scopoletin molecular filter template in negative ionization mode. (C) Mass defect

plots of urine from scopoletin-treated mice. This mass defect filter was performed using sulfate scopoletin molecular as the filter template in negative ionization mode. The potential metabolite points were labeled in mass defect. (D) The chromatograms of scopoletin metabolites in urine filtered by mass defect filter technique with hydroxylation and sulfate scopoletin molecular filter template in negative ionization mode.

Figure S7. Metabolomics analysis of esculetin metabolites. (A) OPLS-DA score plots analysis of feces between vehicle treatment mice and esculetin treatment mice (in positive ionization mode). (B) S-plots observed in OPLS-DA analysis of feces between vehicle-treated group and esculetin-treated group (in positive ionization mode). The potential metabolites were marked in the S-plots. (C) OPLS-DA score plots analysis of plasma between vehicle treatment mice and esculetin treatment mice (in negative ionization mode). (D) S-plots observed in OPLS-DA analysis of plasma between vehicle-treated group and esculetin-treated group (in negative ionization mode). The potential metabolites were marked in the S-plots.

Figure S8. MMDF analysis of esculetin metabolites. (A) Mass defect plots of urine from esculetin-treated mice. This mass defect filter was performed using glucuronate esculetin molecular as the filter template in negative ionization mode. The potential metabolite points were labeled in mass defect. (B) The chromatograms of esculetin metabolites in urine filtered by mass defect filter technique with glucuronate esculetin molecular filter template in negative ionization mode. (C) Mass defect plots of urine from esculetin-treated mice. This mass defect filter was performed using sulfate

esculetin molecular as the filter template in negative ionization mode. The potential metabolite points were labeled in mass defect. (D) The chromatograms of esculetin metabolites in urine filtered by mass defect filter technique with sulfate esculetin molecular filter template in negative ionization mode.

Fig.S1

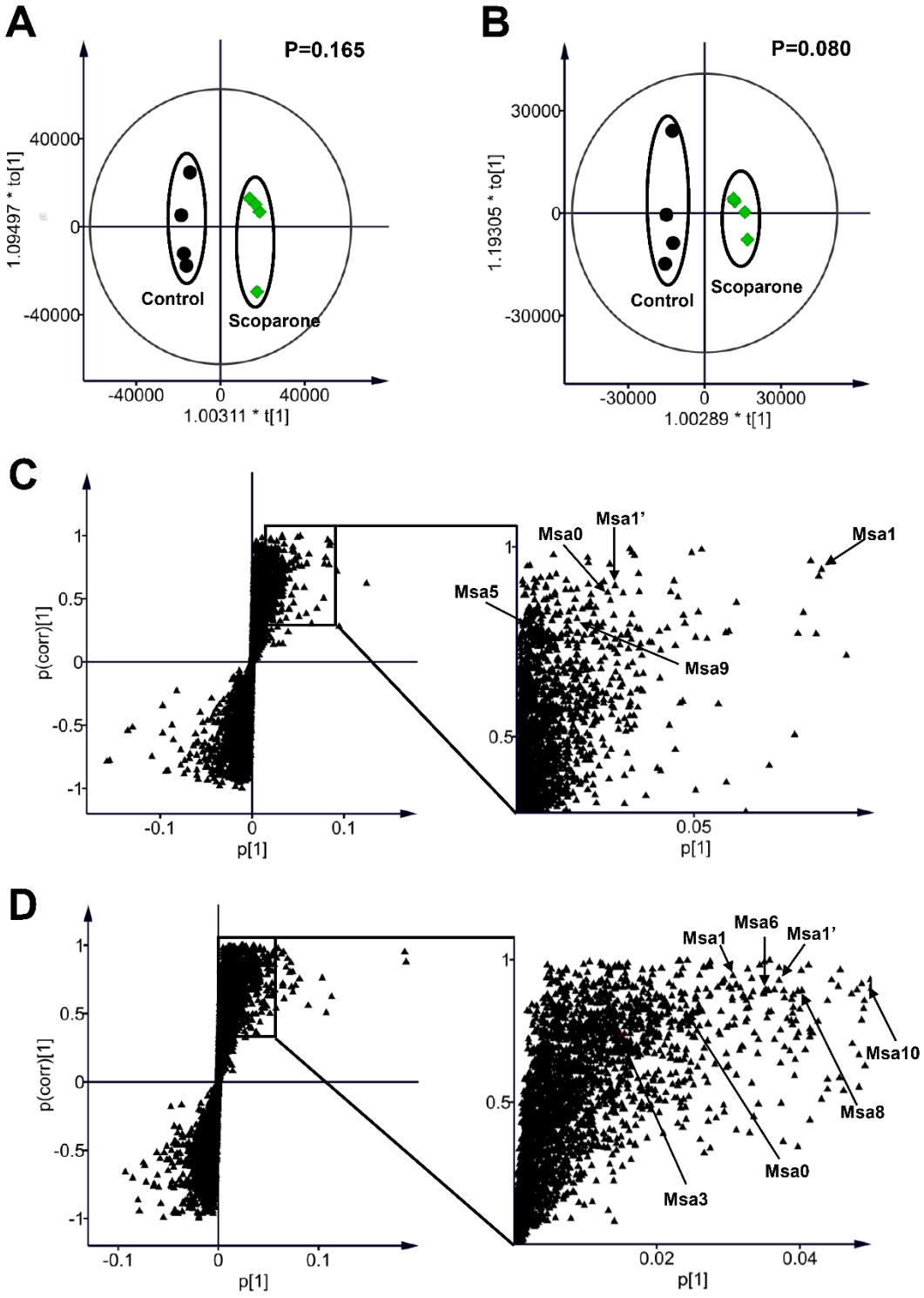
A
P=0.165
1.09497 * to[1]
40000
0
-40000
Control
Scoparone
-40000
0
40000
1.00311 * t[1]
B
P=0.080
1.19305 * to[1]
30000
0
-30000
Control
Scoparone
-30000
0
30000
1.00289 * t[1]
C
p(corr)[1]
1
0.5
0
-0.5
-1
-0.1
0
0.1
p[1]
Msa0
Msa1'
Msa1
Msa5
Msa9
1
0.5
0.05
p[1]
D
p(corr)[1]
1
0.5
0
-0.5
-1
-0.1
0
0.1
p[1]
Msa1
Msa6
Msa1'
Msa10
Msa8
Msa0
Msa3
1
0.5
0.02
0.04
p[1]

Fig. S2

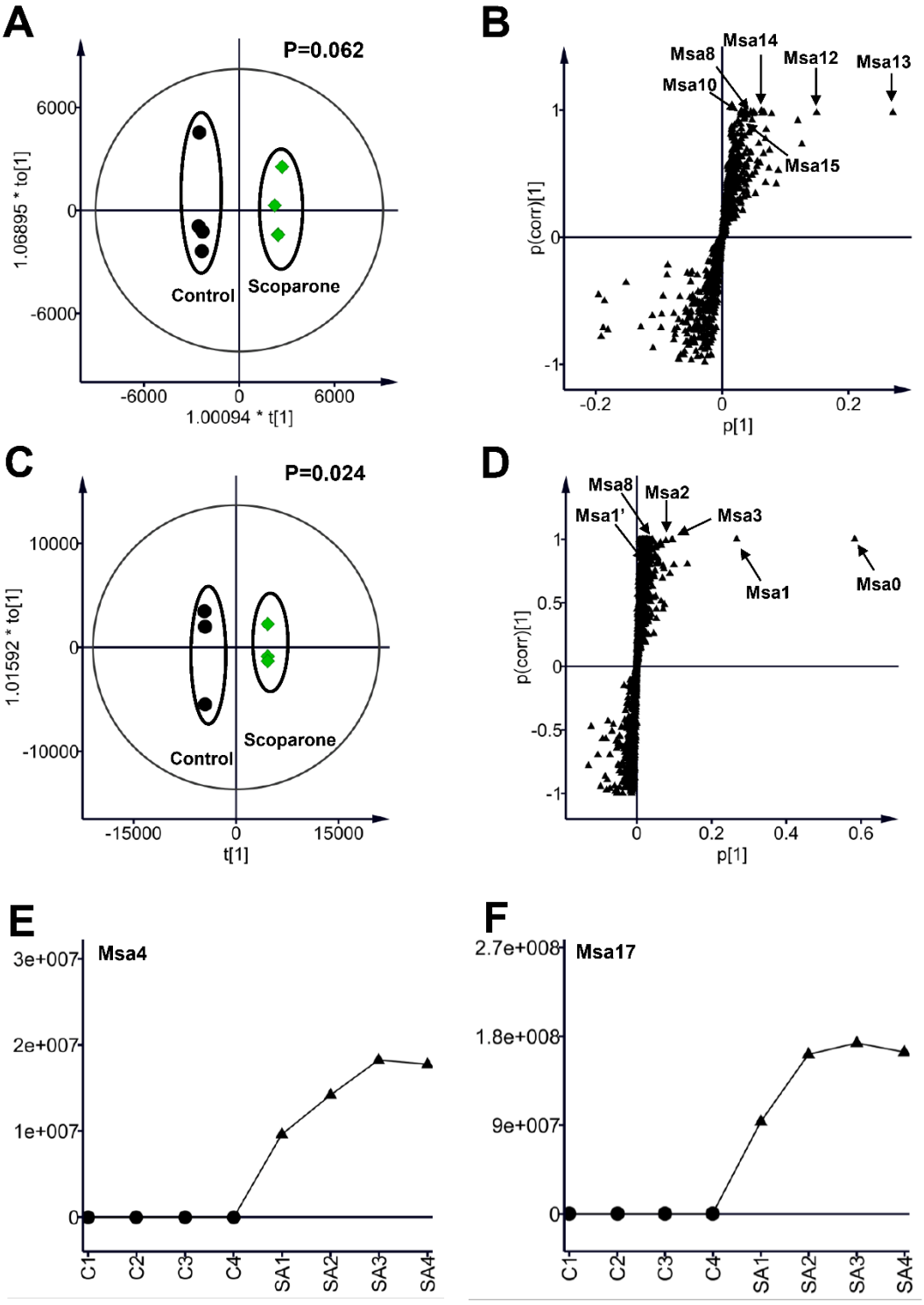
A
P=0.062
6000
0
-6000
1.06895 * to[1]
-6000
0
6000
1.00094 * t[1]
Control
Scoparone
B
Msa8
Msa14
Msa12
Msa13
Msa10
Msa15
1
0
-1
p(corr)[1]
-0.2
0
0.2
p[1]
C
P=0.024
10000
0
-10000
1.01592 * to[1]
-15000
0
15000
t[1]
Control
Scoparone
D
Msa8
Msa2
Msa1'
Msa3
Msa1
Msa0
1
0.5
0
-0.5
-1
p(corr)[1]
0
0.2
0.4
0.6
p[1]
E
Msa4
3e+007
2e+007
1e+007
0
C1
C2
C3
C4
SA1
SA2
SA3
SA4
F
Msa17
2.7e+008
1.8e+008
9e+007
0
C1
C2
C3
C4
SA1
SA2
SA3
SA4

Fig. S3

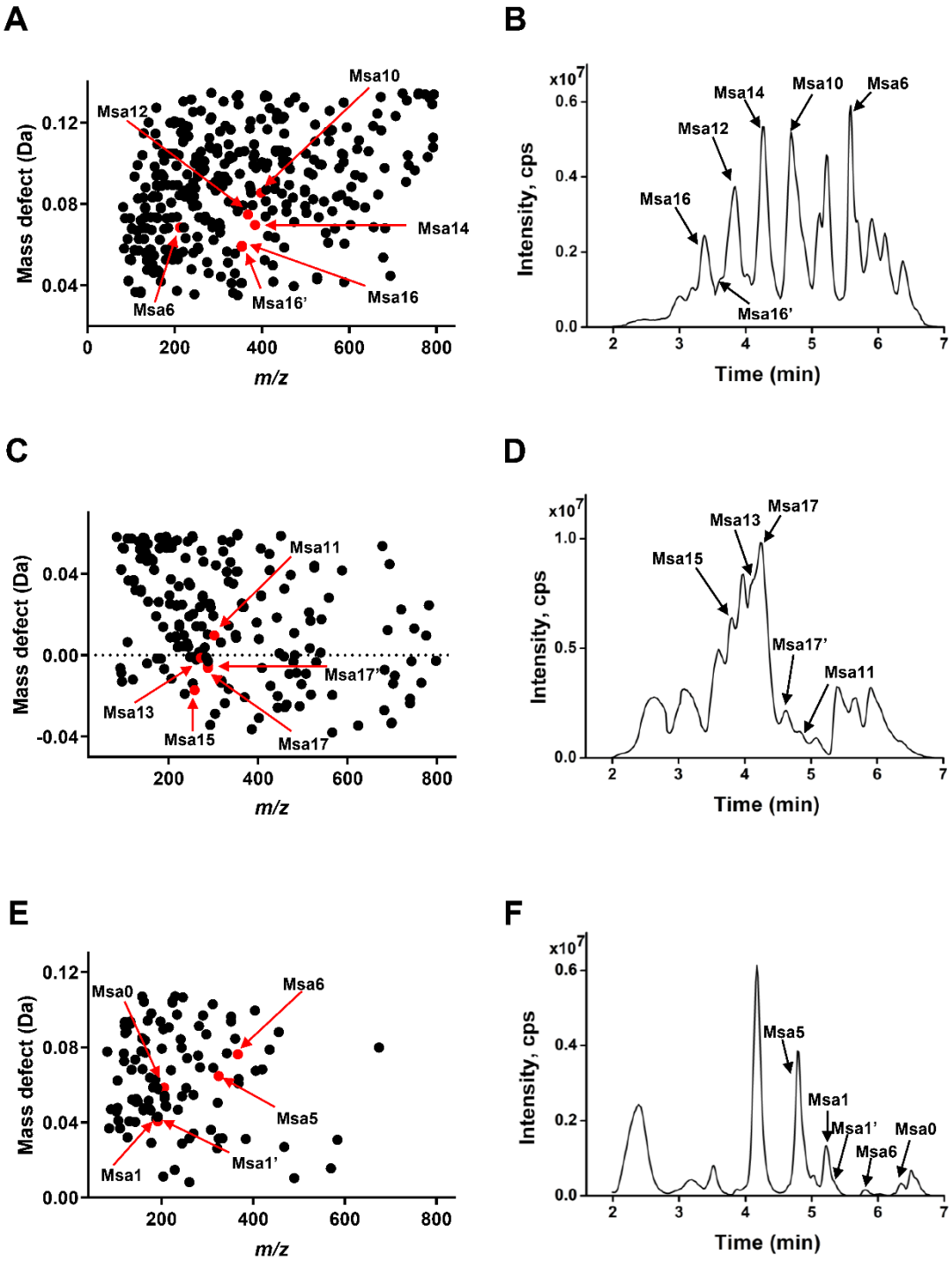
A
Mass defect (Da)
m/z
Msa10
Msa12
Msa14
Msa16
Msa16'
Msa6
B
Intensity, cps
Time (min)
x10^7
Msa14
Msa10
Msa6
Msa12
Msa16
Msa16'
C
Mass defect (Da)
m/z
Msa11
Msa17'
Msa13
Msa15
Msa17
D
Intensity, cps
Time (min)
x10^7
Msa17
Msa13
Msa15
Msa17'
Msa11
E
Mass defect (Da)
m/z
Msa0
Msa6
Msa5
Msa1
Msa1'
F
Intensity, cps
Time (min)
x10^7
Msa5
Msa1
Msa1'
Msa0
Msa6

Fig. S4

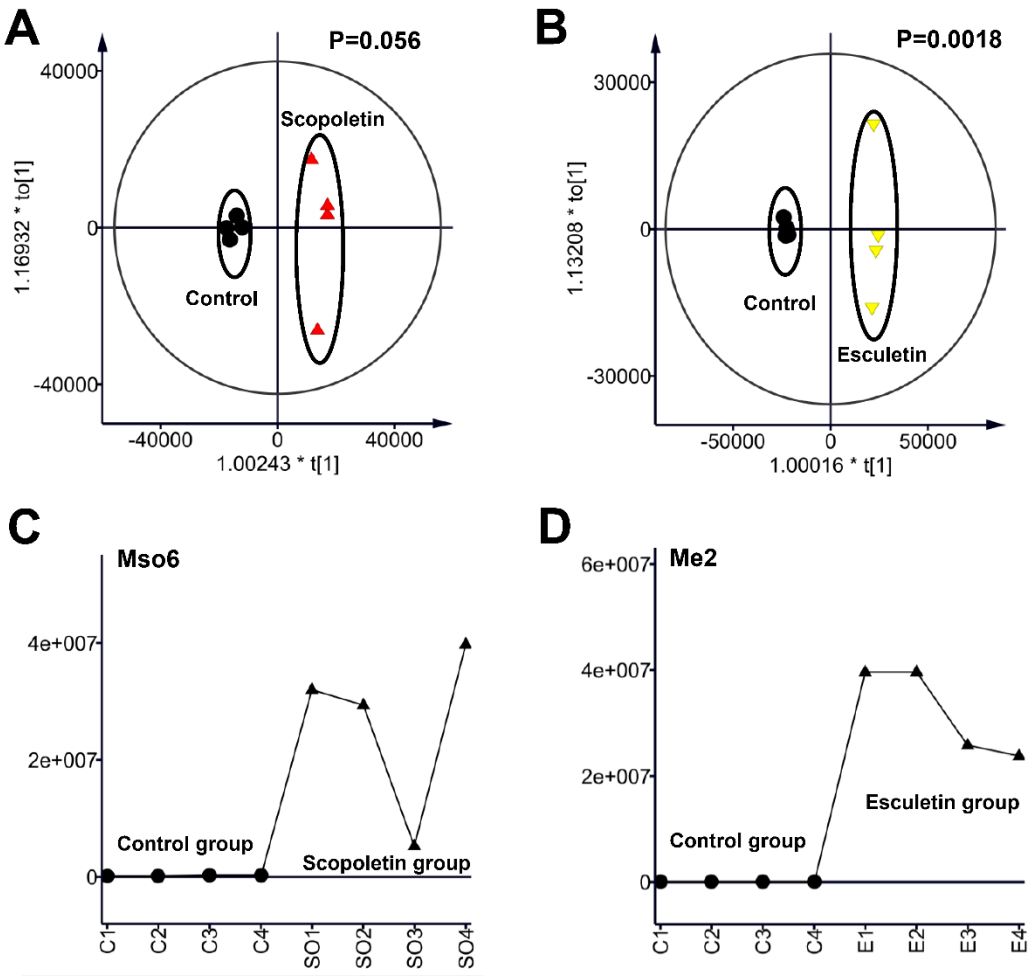
A
P=0.056
Scopoletin
Control
1.16932 * to[1]
1.00243 * t[1]
40000
0
-40000
B
P=0.0018
Control
Esculetin
1.13208 * to[1]
1.00016 * t[1]
30000
-30000
-50000
50000
C
Mso6
4e+007
2e+007
Control group
Scopoletin group
C1
C2
C3
C4
SO1
SO2
SO3
SO4
D
Me2
6e+007
Esculetin group
Control group
E1
E2
E3
E4

Fig. S5

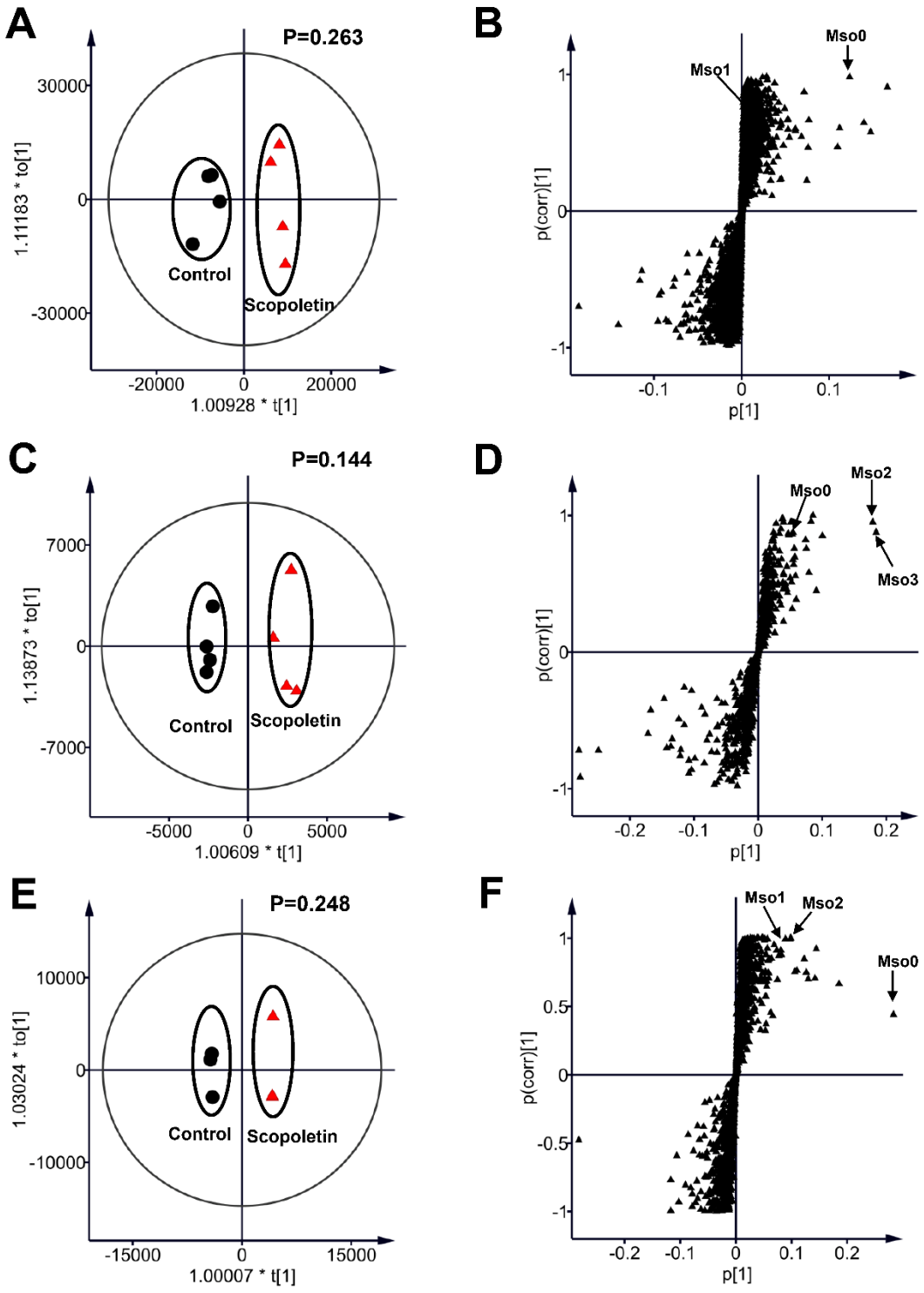
A
P=0.263
30000
0
-30000
1.11183 * to[1]
Control
Scopoletin
-20000
0
20000
1.00928 * t[1]
B
Mso0
Mso1
1
0
-1
p(corr)[1]
-0.1
0
0.1
p[1]
C
P=0.144
7000
0
-7000
1.13873 * to[1]
Control
Scopoletin
-5000
0
5000
1.00609 * t[1]
D
Mso2
Mso0
Mso3
1
0
-1
p(corr)[1]
-0.2
-0.1
0
0.1
0.2
p[1]
E
P=0.248
10000
0
-10000
1.03024 * to[1]
Control
Scopoletin
-15000
0
15000
1.00007 * t[1]
F
Mso1
Mso2
Mso0
1
0.5
0
-0.5
-1
p(corr)[1]
-0.2
-0.1
0
0.1
0.2
p[1]

Fig. S6

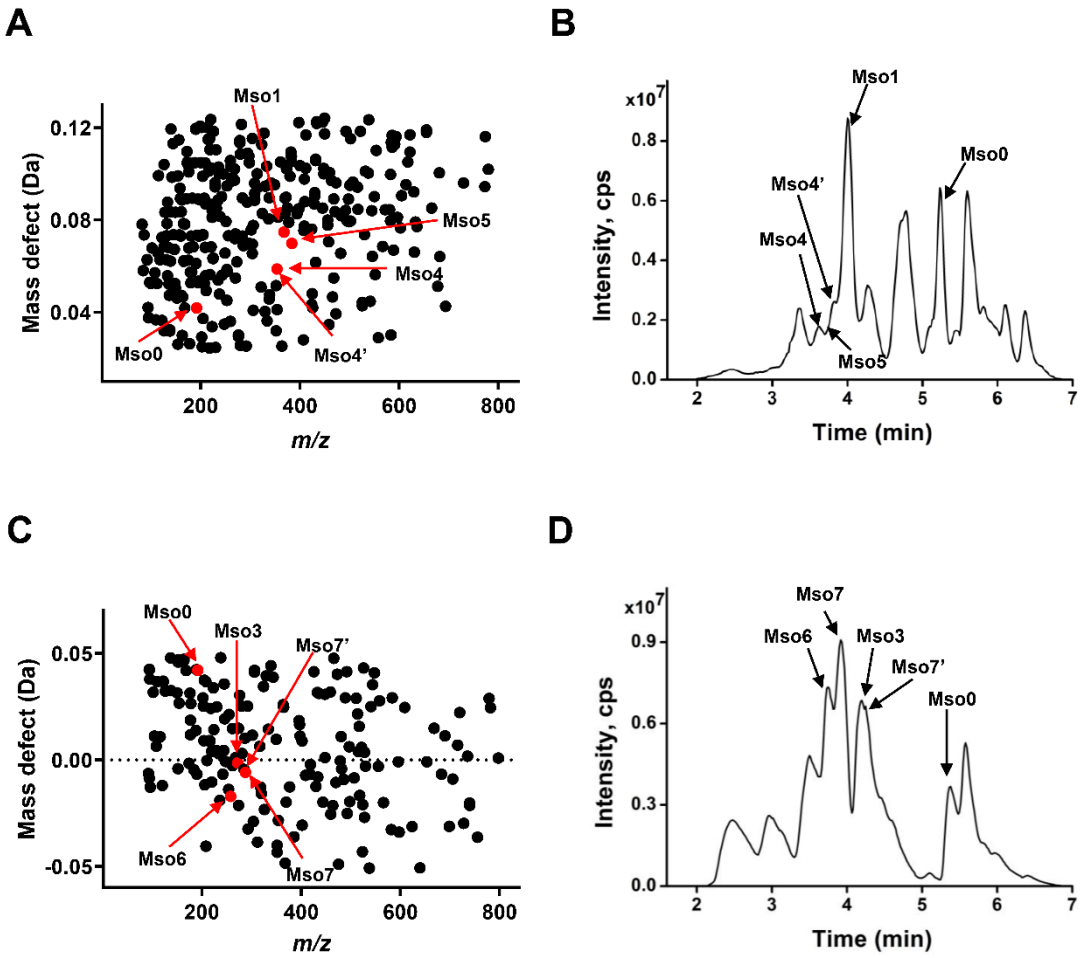
A
Mso1
Mso5
Mso4
Mso0
Mso4'
Mass defect (Da)
0.12
0.08
0.04
200
400
600
800
m/z
B
x10^7
Mso1
Mso0
Mso4'
Mso4
Mso5
Intensity, cps
0.8
0.6
0.4
0.2
0.0
2
3
4
5
6
7
Time (min)
C
Mso0
Mso3
Mso7'
Mso6
Mso7
Mass defect (Da)
0.05
0.00
-0.05
200
400
600
800
m/z
D
x10^7
Mso7
Mso6
Mso3
Mso7'
Mso0
Intensity, cps
0.9
0.6
0.3
0.0
2
3
4
5
6
7
Time (min)

Fig. S7

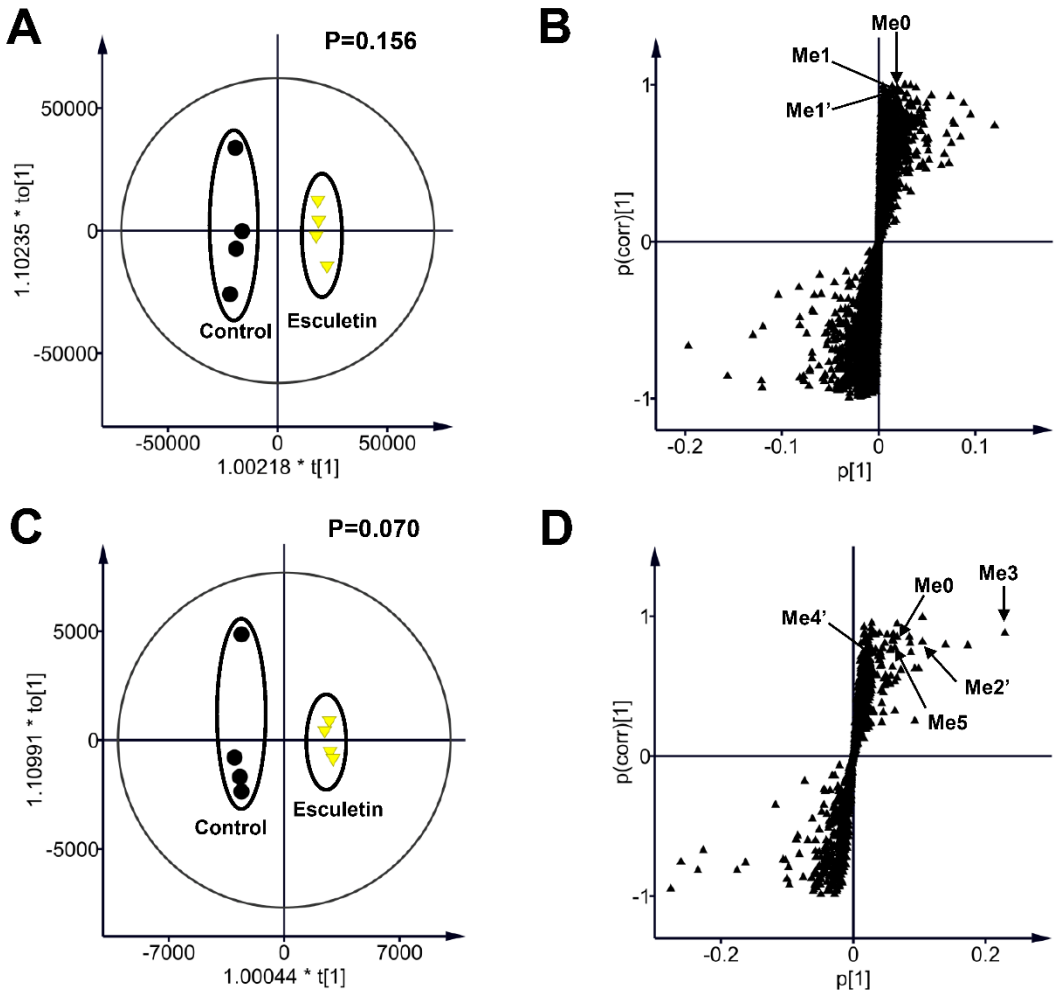
A
P=0.156
50000
0
-50000
1.10235 * to[1]
-50000
0
50000
1.00218 * t[1]
Control
Esculetin
B
Me0
Me1
Me1'
1
0
-1
p(corr)[1]
-0.2
-0.1
0
0.1
p[1]
C
P=0.070
5000
0
-5000
1.10991 * to[1]
-7000
0
7000
1.00044 * t[1]
Control
Esculetin
D
Me0
Me3
Me4'
Me2'
Me5
1
0
-1
p(corr)[1]
-0.2
0
0.2
p[1]

Fig. S8

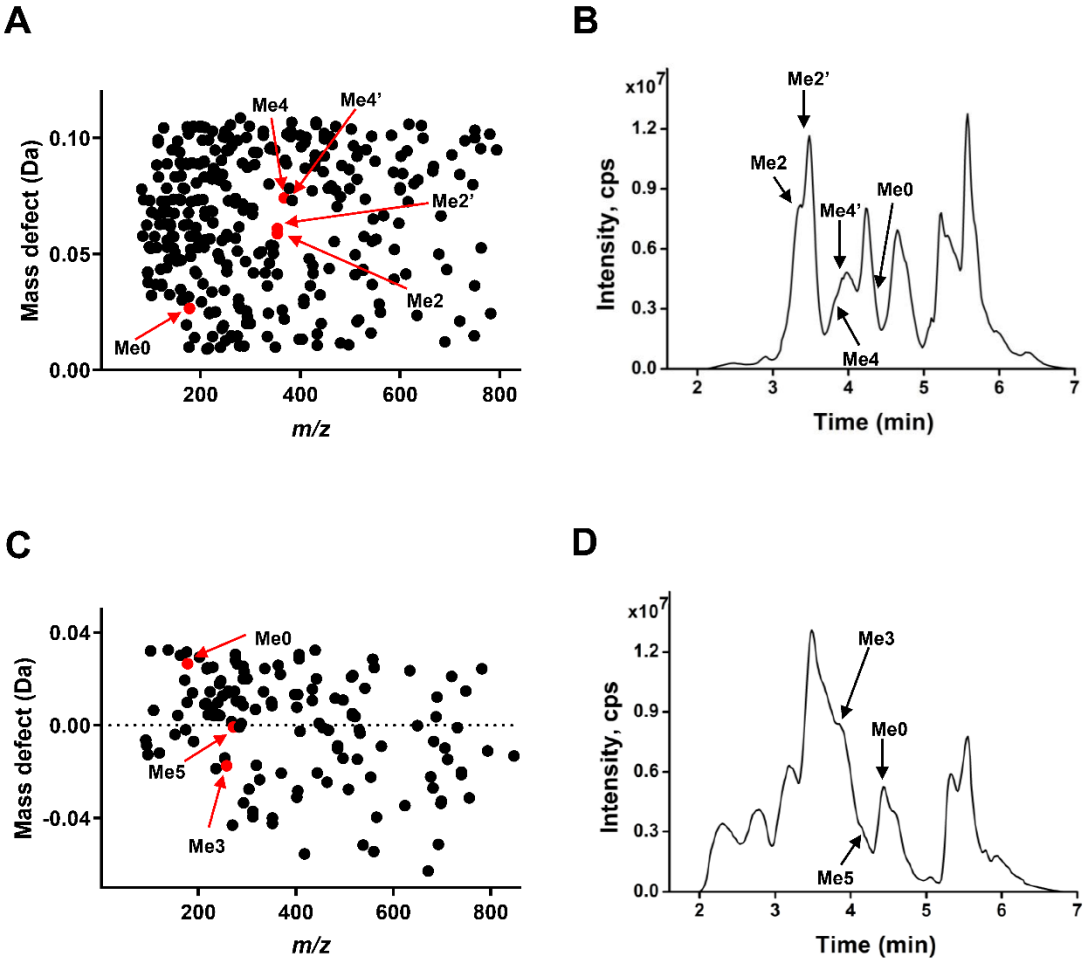
A
Mass defect (Da)
0.10
0.05
0.00
200
400
600
800
m/z
Me4
Me4'
Me2'
Me2
Me0
B
x10^7
Intensity, cps
1.2
0.9
0.6
0.3
0.0
2
3
4
5
6
7
Time (min)
Me2'
Me2
Me4'
Me0
Me4
C
Mass defect (Da)
0.04
0.00
-0.04
200
400
600
800
m/z
Me0
Me5
Me3
D
x10^7
Intensity, cps
1.2
0.9
0.6
0.3
0.0
2
3
4
5
6
7
Time (min)
Me3
Me0
Me5

Table S1. MS/MS fragment ions of scoparone, scopoletin and esculetin metabolites.

Metabolite ID	Formula	Observed *m/z*	Major MS/MS fragment ions (*m/z*)				
			Scoparone metabolism				
Msa0	$C_{11}H_{10}O_4[H^+]$	207.0642	193(-CH_2)	179(-2CH_2)	163(-2CH_2-O)	147(-2CH_2-2O)	151(-2CH_2-CO)
Msa1	$C_{10}H_8O_4[H^+]$	193.0481	179(-CH_2)	147(-CH_2-2O)	165(-CO)	149(-CO_2)	135(-CO_2-CH_2)
Msa1'	$C_{10}H_8O_4[H^+]$	193.0483	179(-CH_2)	147(-CH_2-2O)	165(-CO)	149(-CO_2)	135(-CO_2-CH_2)
Msa2	$C_{11}H_{10}O_5[H^+]$	223.0602	209(-CH_2)	193(-CH_2-O)	163(-CH_2-O-CH_2O)		
Msa3	$C_{10}H_8O_5[H^+]$	209.0472	193(-O)	163(-O-CH_2O)			
Msa4	$C_{11}H_{12}O_5[H^+]$	225.0772	207(-H_2O)	193(-H_2O-CH_2)	179(-H_2O-CO)	163(-H_2O-CO-O)	
Msa5	$C_{14}H_{15}NO_6S[H^+]$	326.0719	280(-CH_2O_2)	237(-CH_2O_2-C_2H_5N)	207(-$C_3H_5NO_2S$)		
Msa6	$C_{16}H_{17}NO_7S[H^+]$	368.0813	326(-C_2H_2O)	280(-C_2H_2O-CH_2O_2)	237(-C_2H_2O-CH_2O_2-C_2H_5N)	207(-$C_5H_7NO_3S$)	
Msa7	$C_{10}H_{14}O_4[H^+]$	199.0965	181(-H_2O)	163(-2H_2O)	149(-2H_2O-CH_2)		
Msa8	$C_{10}H_{12}O_5[H^-]$	211.0614	181(-CH_2O)	167(-CH_2O-CH_2)			
Msa9	$C_9H_6O_4[H^-]$	177.0191	149(-CO)	133(-CO-O)	105(-CO-O-CO)		
Msa10	$C_{17}H_{18}O_{11}[H^-]$	397.0780	221(-$C_6H_8O_6$)	206(-$C_6H_8O_6$-CH_2)	175(-$C_{11}H_{10}O_5$)	113(-$C_{11}H_{10}O_5$-CH_2O_3)	95(-$C_{11}H_{10}O_5$-CH_2O_3-H_2O)
Msa11	$C_{11}H_{10}O_8S[H^-]$	301.0028	221(-SO_3)	206(-SO_3-CH_2)	178(-SO_3-CH_2-CO)	97(-$C_{11}H_8O_4$)	
Msa12	$C_{16}H_{16}O_{10}[H^-]$	367.0672	191(-$C_6H_8O_6$)	175(-$C_{10}H_8O_4$)	113(-$C_{10}H_8O_4$-CH_2O_3)	95(-$C_{10}H_8O_4$-CH_2O_3-H_2O)	
Msa13	$C_{10}H_8O_7S[H^-]$	270.9914	191(-SO_3)	176(-SO_3-O)	148(-SO_3-O-CO)	120(-SO_3-O-2CO)	97(-$C_{10}H_6O_3$)
Msa14	$C_{16}H_{16}O_{11}[H^-]$	383.0614	207(-$C_6H_8O_6$)	192(-$C_6H_8O_6$-O)	175(-$C_{10}H_8O_5$)	113(-$C_{10}H_8O_5$-CH_2O_3)	95(-$C_{10}H_8O_5$-CH_2O_3-H_2O)
Msa15	$C_9H_6O_7S[H^-]$	256.9754	177(-SO_3)	149(-SO_3-CO)	133(-SO_3-CO-O)	105(-SO_3-CO-O-CO)	97(-$C_9H_4O_3$)
Msa16	$C_{15}H_{14}O_{10}[H^-]$	353.0533	177(-$C_6H_8O_6$)	133(-$C_6H_8O_6$-CO_2)	175(-$C_9H_6O_4$)	113(-$C_9H_6O_4$-CH_2O_3)	95(-$C_9H_6O_4$-CH_2O_3-H_2O)
Msa16'	$C_{15}H_{14}O_{10}[H^-]$	353.0502	177(-$C_6H_8O_6$)	133(-$C_6H_8O_6$-CO_2)	175(-$C_9H_6O_4$)	113(-$C_9H_6O_4$-CH_2O_3)	95(-$C_9H_6O_4$-CH_2O_3-H_2O)
Msa17	$C_{10}H_8O_8S[H^-]$	286.9869	207(-SO_3)	192(-SO_3-O)	162(-SO_3-O-CH_2O)	146(-SO_3-O-CH_2O-O)	97(-$C_{10}H_6O_4$)
Msa17'	$C_{10}H_8O_8S[H^-]$	286.9872	207(-SO_3)	192(-SO_3-O)	162(-SO_3-O-CH_2O)	146(-SO_3-O-CH_2O-O)	97(-$C_{10}H_6O_4$)

Table S1. *Continued*

Metabolite ID	Formula	Observed *m/z*	Major MS/MS fragment ions (*m/z*)				
			Scopoletin metabolism				
Mso0	$C_{10}H_8O_4[H^-]$	191.0349	176(-CH_2)	148(-CH_2-CO)	134(-CH_2-CO-O)	120(-CH_2-CO-O-CH_2)	104(-CH_2-CO-O-CH_2-O)
Mso1	$C_9H_6O_4[H^-]$	177.0191	149(-CO)	133(-CO-O)	105(-CO-O-CO)		
Mso2	$C_{10}H_8O_5[H^-]$	207.0295	191(-O)	161(-O-CH_2O)			
Mso3	$C_{16}H_{16}O_{10}[H^-]$	367.0668	191(-$C_6H_8O_6$)	177(-$C_6H_8O_6$-CH_2)	175(-$C_{10}H_8O_4$)	113(-$C_{10}H_8O_4$-CH_2O_3)	95(-$C_{10}H_8O_4$-CH_2O_3-H_2O)
Mso4	$C_{10}H_8O_7S[H^-]$	270.9913	191(-SO_3)	176(-SO_3-O)	148(-SO_3-O-CO)	120(-SO_3-O-2CO)	97(-$C_{10}H_6O_3$)
Mso5	$C_{15}H_{14}O_{10}[H^-]$	353.0518	177(-$C_6H_8O_6$)	133(-$C_6H_8O_6$-CO_2)	175(-$C_9H_6O_4$)	113(-$C_9H_6O_4$-CH_2O_3)	95(-$C_9H_6O_4$-CH_2O_3-H_2O)
Mso5'	$C_{15}H_{14}O_{10}[H^-]$	353.0516	177(-$C_6H_8O_6$)	133(-$C_6H_8O_6$-CO_2)	175(-$C_9H_6O_4$)	113(-$C_9H_6O_4$-CH_2O_3)	95(-$C_9H_6O_4$-CH_2O_3-H_2O)
Mso6	$C_{16}H_{16}O_{11}[H^-]$	383.0627	207(-$C_6H_8O_6$)	192(-$C_6H_8O_6$-O)	175(-$C_{10}H_8O_5$)	113(-$C_{10}H_8O_5$-CH_2O_3)	95(-$C_{10}H_8O_5$-CH_2O_3-H_2O)
Mso7	$C_9H_6O_7S[H^-]$	256.9756	177(-SO_3)	149(-SO_3-CO)	133(-SO_3-CO-O)	105(-SO_3-CO-O-CO)	97(-$C_9H_4O_3$)
Mso8	$C_{10}H_8O_8S[H^-]$	286.9871	207(-SO_3)	192(-SO_3-O)	162(-SO_3-O-CH_2O)	146(-SO_3-O-CH_2O-O)	97(-$C_{10}H_6O_4$)
Mso8'	$C_{10}H_8O_8S[H^-]$	286.9870	207(-SO_3)	192(-SO_3-O)	176(-SO_3-2O)	162(-SO_3-2O-CH_2)	97(-$C_{10}H_6O_4$)
			Esculetin metabolism				
Me0	$C_9H_6O_4[H^-]$	177.0192	149(-CO)	133(-CO-O)	105(-CO-O-CO)	93(-CO-O-CO-C)	77(-CO-O-CO-C-O)
Me1	$C_{10}H_8O_4[H^+]$	193.0496	179(-CH_2)	147(-CH_2-2O)	165(-CO)	149(-CO_2)	
Me1'	$C_{10}H_8O_4[H^+]$	193.0483	179(-CH_2)	147(-CH_2-2O)	165(-CO)	149(-CO_2)	
Me2	$C_{15}H_{14}O_{10}[H^-]$	353.0517	177(-$C_6H_8O_6$)	133(-$C_6H_8O_6$-CO_2)	175(-$C_9H_6O_4$)	113(-$C_9H_6O_4$-CH_2O_3)	95(-$C_9H_6O_4$-CH_2O_3-H_2O)
Me2'	$C_{15}H_{14}O_{10}[H^-]$	353.0508	177(-$C_6H_8O_6$)	133(-$C_6H_8O_6$-CO_2)	175(-$C_9H_6O_4$)	113(-$C_9H_6O_4$-CH_2O_3)	95(-$C_9H_6O_4$-CH_2O_3-H_2O)
Me3	$C_9H_6O_7S[H^-]$	256.9757	177(-SO_3)	149(-SO_3-CO)	133(-SO_3-CO-O)	105(-SO_3-CO-O-CO)	97(-$C_9H_4O_3$)
Me4	$C_{16}H_{16}O_{10}[H^-]$	367.0679	191(-$C_6H_8O_6$)	177(-$C_6H_8O_6$-CH_2)	175(-$C_{10}H_8O_4$)	113(-$C_{10}H_8O_4$-CH_2O_3)	95(-$C_{10}H_8O_4$-CH_2O_3-H_2O)
Me4'	$C_{16}H_{16}O_{10}[H^-]$	367.0663	191(-$C_6H_8O_6$)	177(-$C_6H_8O_6$-CH_2)	175(-$C_{10}H_8O_4$)	113(-$C_{10}H_8O_4$-CH_2O_3)	95(-$C_{10}H_8O_4$-CH_2O_3-H_2O)
Me5	$C_{10}H_8O_7S[H^-]$	270.9918	191(-SO_3)	176(-SO_3-O)	148(-SO_3-O-CO)	120(-SO_3-O-2CO)	97(-$C_{10}H_6O_3$)

Table S2. Common metabolic reactions and corresponding metabolites in urine of mice following treatment of three compounds.

Metabolic reaction	Scoparone	Scopoletin	Esculetin
Demethylation	Msa1, Msa1', Msa3, Msa9, Msa12, Msa13, Msa14, Msa15, Msa16, Msa16', Msa17, Msa17'	Mso1, Mso5, Mso5', Mso7	
Hydroxylation	Msa3, Msa10, Msa11, Msa14, Msa17, Msa17'	Mso6, Mso8, Mso8'	
Glucuronic acid conjugation	Msa10, Msa12, Msa14, Msa16, Msa16'	Mso3, Mso5, Mso5', Mso6	Me2, Me2', Me4, Me4'
Sulfate acid conjugation	Msa11, Msa13, Msa15, Msa17, Msa17'	Mso4, Mso7, Mso8, Mso8'	Me3, Me5

Table S3. Common metabolic reactions and corresponding metabolites of scoparone and scopoletin in MLM.

Metabolic reaction	Scoparone	Scopoletin
Demethylation	Msa1, Msa1', Msa3	Mso1
Hydroxylation	Msa2, Msa3	Mso2