

Human gastrointestinal metabolism of Cistanches Herba water extract *in vitro*: elucidation of the metabolic profile based on comprehensive metabolites identification in gastric juice, intestinal juice, human intestinal bacteria and intestinal microsome

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Table S1. Percentage of Relative Content and the Area of Prototype Components of *C. deserticola* in Different Samples.

No.	Name	CD ^a (%) (area abs)	GJ ^b (%) (area abs)	IJ ^c (%) (area abs)	HIMP1 ^d (%) (area abs)	HIMP2 ^e (%) (area abs)	HIB ^f (%) (area abs)
C1	geniposidic acid	0.71 (35.45)	0.62 (44.34)	0.06 (2.28)	0.00 (0.00)	0.00 (0.00)	0.22 (10.46)
C3	8-epiloganic acid	8.60 (431.87)	6.96 (496.60)	1.11 (39.31)	0.35 (17.72)	0.52 (15.33)	3.32 (161.19)
C4	decaffeoylacteoside	1.88 (94.56)	1.34 (95.72)	4.59 (161.96)	0.69 (35.24)	1.48 (43.95)	1.62 (78.75)
C5	cistanoside F	0.23 (11.41)	0.39 (28.02)	0.59 (20.81)	0.24 (12.42)	0.66 (19.53)	0.09 (4.59)
C6	salidroside	0.12 (6.20)	0.05 (3.33)	0.19 (6.57)	0.08 (3.96)	0.11 (3.36)	0.00 (0.20)
C7	cistanoside E	0.50 (25.03)	0.38 (26.98)	3.83 (134.97)	0.00 (0.00)	1.00 (29.63)	0.41 (19.69)
C8	cistantubuloside C ₁ /C ₂	0.15 (7.40)	0.06 (4.23)	0.11 (3.81)	0.05 (2.66)	0.22 (6.48)	0.18 (8.79)
C9	cistanoside H	1.38 (69.50)	1.29 (92.20)	14.49 (510.88)	1.23 (62.64)	1.57 (46.77)	2.21 (107.34)
C10	echinacoside	20.22 (1014.86)	21.99 (1568.14)	34.85 (1228.97)	16.21 (826.54)	17.01 (505.61)	15.70 (761.45)
C11	poliumoside	0.69 (34.79)	0.72 (51.64)	0.79 (27.69)	0.53 (27.16)	0.74 (22.02)	0.67 (32.59)
C12	cistanoside A	10.64 (534.25)	7.38 (526.66)	13.56 (478.28)	10.79 (549.88)	16.28 (483.96)	10.64 (515.94)
C13	kankanoside A or isomer	0.63 (31.53)	0.63 (45.18)	0.70 (24.80)	0.29 (14.59)	0.01 (0.38)	0.00 (0.00)
C14	acteoside	9.75 (489.43)	11.61 (827.91)	7.88 (278.04)	7.76 (395.43)	7.15 (212.64)	11.53 (559.10)
C15	kankanoside A or isomer	1.00 (50.09)	0.90 (64.18)	1.10 (38.78)	0.49 (24.85)	1.19 (35.47)	0.00 (0.00)
C16	tubuloside A	0.56 (28.34)	0.53 (37.97)	0.90 (31.59)	0.04 (1.90)	0.49 (14.70)	0.58 (28.29)
C17	isoacteoside	16.24 (815.19)	17.77 (1267.22)	0.01 (0.23)	22.29 (1136.13)	16.13 (479.40)	19.92 (966.11)

C18	kankanoside N		3.12 (156.70)	0.08 (5.94)	4.22 (148.75)	2.27 (115.55)	4.15 (123.37)	0.03 (1.32)
C19	syringalide	A	0.83 (41.69)	1.03 (73.71)	1.30 (45.79)	1.21 (61.82)	0.90 (26.78)	1.09 (52.88)
	-3'-a-L-rhamnopyranoside							
C21	cistanoside C		1.46 (73.14)	1.68 (119.59)	2.63 (92.61)	1.93 (98.63)	2.43 (72.21)	1.68 (81.68)
C22	isomer of syringalide	A	1.13 (56.55)	0.00 (0.00)	0.00 (0.00)	0.10 (5.05)	1.28 (38.02)	1.21 (58.47)
	-3'-a-L-rhamnopyranoside							
C23	2'-acetylacteoside		4.04 (202.88)	4.60 (327.78)	5.51 (194.41)	4.25 (216.85)	2.95 (87.64)	5.40 (262.00)
C24	isocistanoside C		3.03 (151.94)	2.65 (188.85)	0.01 (0.53)	7.27 (370.82)	6.13 (182.17)	3.48 (169.01)
C25	osmanthuside B or osmanthuside B6		0.36 (18.23)	0.48 (34.50)	0.83 (29.34)	0.70 (35.62)	0.85 (25.38)	0.26 (12.80)
C26	tubuloside B		9.95 (499.29)	14.04 (1001.62)	0.00 (0.00)	16.49 (840.41)	11.82 (351.33)	16.27 (789.08)
C27	osmanthuside B or osmanthuside B6		0.68 (34.37)	0.89 (63.31)	0.00 (0.00)	1.31 (66.98)	1.52 (45.09)	0.74 (35.71)
C28	salsaside F or isomer		0.15 (7.54)	0.15 (10.75)	0.26 (9.14)	0.23 (11.81)	0.11 (3.33)	0.25 (11.99)
C29	cistanoside D		0.24 (12.00)	0.28 (19.84)	0.36 (12.87)	0.29 (14.57)	0.46 (13.56)	0.32 (15.58)
C30	salsaside F or isomer		0.57 (28.81)	0.70 (49.94)	0.03 (0.92)	0.91 (46.14)	0.43 (12.66)	0.53 (25.77)
C31	isocistanoside D		0.58 (29.18)	0.00 (0.00)	0.10 (3.44)	1.11 (56.59)	1.34 (39.85)	0.70 (33.80)
C32	cistanoside K or cistansinenside A or salsaside E or isomer		0.55 (27.60)	0.78 (55.62)	0.00 (0.00)	0.90 (45.99)	1.07 (31.89)	0.95 (45.87)
Sum			100.00 (5019.85)	100.00 (7131.75)	100.00 (3526.78)	100.00 (5097.97)	100.00 (2972.49)	100.00 (4850.47)

^a CD: *C. deserticola*, ^b GJ: gastric juice, ^c IJ: intestinal juice, ^d HIMP1: human intestinal microsome phase I, ^e HIMP2: human intestinal microsome phase II, ^f HIB: human intestinal bacteria.

Table S2. Percentage of Relative Content and the Area of Prototype Components of *C. tubulosa* in Different Samples.

No.	Name	CT ^a (%) (area abs)	GJ ^b (%) (area abs)	IJ ^c (%) (area abs)	HIMP1 ^d (%) (area abs)	HIMP2 ^e (%) (area abs)	HIB ^f (%) (area abs)
C1	geniposidic acid	7.96 (379.53)	7.70 (263.07)	1.45 (24.58)	0.66 (30.09)	1.77 (55.52)	1.27 (17.72)
C2	kankanose	4.93 (235.07)	1.77 (60.48)	1.71 (29.12)	2.18 (99.19)	5.57 (174.97)	0.36 (5.02)
C3	8-epiloganic acid	18.14 (865.03)	11.93 (407.81)	2.66 (45.17)	1.23 (56.11)	3.37 (105.85)	4.23 (59.02)
C4	decaffeoylacteoside	1.44 (68.79)	0.50 (17.10)	11.64 (197.88)	1.08 (49.29)	1.33 (41.75)	0.59 (8.18)
C5	cistanoside F	0.54 (25.76)	0.00 (0.00)	1.66 (28.14)	2.01 (91.13)	2.84 (89.10)	0.45 (6.26)
C6	salidroside	0.06 (2.94)	0.07 (2.38)	0.10 (1.77)	0.21 (9.51)	0.26 (8.01)	0.00 (0.00)
C8	cistantubuloside C ₁ /C ₂	1.52 (72.45)	1.85 (63.20)	3.60 (61.19)	3.74 (169.89)	6.35 (199.55)	2.35 (32.81)
C9	cistanoside H	0.05 (2.47)	0.01 (0.40)	0.43 (7.35)	0.08 (3.43)	0.07 (2.18)	0.09 (1.25)
C10	echinacoside	36.42 (1736.70)	43.83 (1497.89)	52.03 (884.22)	35.14 (1596.88)	39.52 (1241.09)	64.81 (904.58)
C11	poliumoside	1.57 (74.77)	4.01 (137.10)	6.81 (115.69)	2.39 (108.52)	8.15 (256.03)	1.95 (27.24)
C12	cistanoside A	0.34 (16.40)	0.17 (5.86)	0.29 (4.89)	0.29 (13.11)	0.15 (4.61)	0.13 (1.85)
C13	kankanoside A or isomer	0.25 (11.87)	0.28 (9.49)	0.27 (4.56)	0.21 (9.46)	0.33 (10.32)	0.00 (0.00)
C14	acteoside	6.59 (314.10)	7.40 (252.76)	9.49 (161.35)	13.96 (634.66)	5.78 (181.62)	7.87 (109.84)
C16	tubuloside A	2.93 (139.50)	2.70 (92.43)	4.49 (76.26)	3.97 (180.52)	3.15 (98.83)	2.33 (32.55)
C17	isoacteoside	14.74 (702.66)	15.45 (528.19)	0.00 (0.00)	26.61 (1209.42)	15.45 (485.17)	12.16 (169.73)
C19	syringalide -3'-a-L-rhamnopyranoside	A 0.16 (7.82)	0.17 (5.92)	0.37 (6.34)	0.66 (29.84)	0.54 (16.91)	0.15 (2.13)

C20	syringaresinol	1.14 (54.31)	1.37 (46.76)	2.71 (46.02)	3.32 (150.86)	4.20 (131.96)	0.03 (0.42)
C23	2'-acetyllacteoside	0.22 (10.69)	0.13 (4.39)	0.20 (3.45)	0.34 (15.58)	0.08 (2.47)	0.48 (6.71)
C25	osmanthuside B or osmanthuside B6	0.02 (0.93)	0.06 (2.15)	0.09 (1.60)	0.19 (8.76)	0.23 (7.13)	0.00 (0.00)
C26	tubuloside B	0.86 (40.85)	0.53 (18.20)	0.00 (0.00)	1.30 (58.89)	0.52 (16.48)	0.66 (9.18)
C27	osmanthuside B or osmanthuside B6	0.10 (4.94)	0.05 (1.85)	0.00 (0.00)	0.41 (18.49)	0.36 (11.19)	0.09 (1.25)
C30	salsaside F or isomer	0.02 (0.93)	0.01 (0.43)	0.00 (0.00)	0.02 (1.05)	0.00 (0.00)	0.00 (0.00)
Sum		100.00 (4768.52)	100.00 (3417.84)	100.00 (1699.60)	100.00 (4544.69)	100.00 (3140.74)	100.00 (1395.73)

^a CT: *C. tubulosa*, ^b GJ: gastric juice, ^c IJ: intestinal juice, ^d HIMP1: human intestinal microsome phase I, ^e HIMP2: human intestinal microsome phase II, ^f HIB: human intestinal bacteria.

Table S3. UPLC-Q-TOF-MS Data of the Identified Metabolites of *C. deserticola* (CD) and *C. tubulosa* (CT) Sequentially Produced by Gastric Juice (GJ), Intestinal Juice (IJ), and Human Intestinal Bacteria (HIB).

No.	t _R (min)	Measured mass (Da)	Error (mDa)	Formula	MS/MS fragment ions (Da)	Source		Identification
						CD	CT	
M1	1.53	315.1064	−1.6	C ₁₄ H ₂₀ O ₈	153.0550, 123.0444	GJ IJ	GJ IJ	3, 4-dihydroxyphenethyl glycoside
M3	1.60	153.0561	0.9	C ₈ H ₁₀ O ₃	123.0465	HIB	HIB	hydroxytyrosol
M4	1.84	163.0400	0.5	C ₉ H ₈ O ₃	135.0448, 119.0487	HIB	HIB	dehydroxylated caffeic acid
M6	2.49	181.0502	0.1	C ₉ H ₁₀ O ₄	137.0600	HIB	HIB	3, 4-dihydroxybenzenepropionic acid
M7	2.58	179.0345	0.1	C ₉ H ₈ O ₄	135.0436	GJ IJ HIB	GJ IJ	caffeic acid
M13	4.06	165.0579	2.7	C ₉ H ₁₀ O ₃	121.0601, 119.0493	HIB	HIB	3-hydroxyphenylpropionic acid
M19	4.53	517.1964	4.3	C ₂₃ H ₃₄ O ₁₃	475.1854, 329.1351	HIB	ND ^a	methylated cistanoside H
M21	4.61	787.2634	−2.7	C ₃₅ H ₄₈ O ₂₀	625.2178	HIB	ND ^a	reduction of echinacoside
M23	5.41	813.2811	−0.6	C ₃₇ H ₅₀ O ₂₀	785.2506, 623.1970, 461.1627, 161.0224	HIB	ND ^a	dimethylated ECH
M25	5.46	477.1419	2.2	C ₂₃ H ₂₆ O ₁₁	179.0339, 161.0231	HIB	ND ^a	calceolarioside A
M32	6.90	651.2271	−1.8	C ₃₁ H ₄₀ O ₁₅	623.1972, 179.0332, 161.0232	HIB	ND ^a	dimethylated acteoside
M33	6.94	185.1186	0.8	C ₁₀ H ₁₈ O ₃	137.0107	HIB	HIB	deglycosylated kankanoside N
M35	7.35	651.2326	3.7	C ₃₁ H ₄₀ O ₁₅	623.1947, 461.1596, 179.0320, 161.0239, 135.0449	HIB	ND ^a	dimethylated acteoside

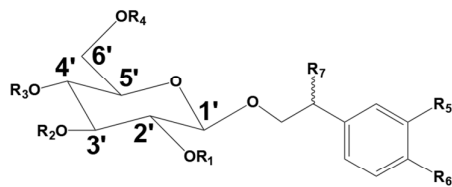
^a ND: not detected.

Figure Captions

Figure S1. The chemical structures of prototype components of *Cistanches Herba* water extract.

Figure S2. The chemical structures of identified metabolites of *Cistanches Herba* water extract produced by gastric juice, intestinal juice, human intestinal bacteria, human intestinal microsome phase I, and human intestinal microsome phase II.

Figure S3. Total ion chromatograms (TIC) of *C. deserticola* water extract (a), bio-transformed *C. deserticola* water extract sequentially by 4 h gastric juice (b), 6 h intestinal juice (c), 24 h human intestinal bacteria (d), and 48 h human intestinal bacteria (e).



No.	Identification	R1	R2	R3	R4	R5	R6	R7
C4	decaffeoyllacteoside	H	Rha	H	H	OH	OH	H
C6	salidroside	H	H	H	H	H	OH	H
C7	cistanoside E	H	Rha	H	H	OMe	OH	H
C8	cistantubuloside C ₁ /C ₂	H	Rha	Cf	Glc	OH	OH	OH
C9	cistanoside H	Ac	Rha	H	H	OH	OH	H
C10	echinacoside	H	Rha	Cf	Glc	OH	OH	H
C11	poliumoside	H	Rha	Cf	Rha	OH	OH	H
C12	cistanoside A	H	Rha	Cf	Glc	OMe	OH	H
C14	acteoside	H	Rha	Cf	H	OH	OH	H
C16	tubuloside A	Ac	Rha	Cf	Glc	OH	OH	H
C17	isoacteoside	H	Rha	H	Cf	OH	OH	H
C19	syringalide A-3'- α -L-rhamnopyranoside	H	Rha	Cf	H	H	OH	H
C21	cistanoside C	H	Rha	Cf	H	OMe	OH	H
C22	isosyringalide A-3'- α -L-rhamnopyranoside	H	Rha	Cm	H	OH	OH	H
C23	2'-acetylacteoside	Ac	Rha	Cf	H	OH	OH	H
C24	isocistanoside C	H	Rha	H	Cf	OMe	OH	H
C25	osmanthuside B or osmanthuside B6	H	Rha	Cm/H	H/Cm	H	OH	H
C26	tubuloside B	Ac	Rha	H	Cf	OH	OH	H
C27	osmanthuside B or osmanthuside B6	H	Rha	Cm/H	H/Cm	H	OH	H
C28	salsaside F or isomer	Ac	Rha	H	Cm	OH	OH	H
C29	cistanoside D	H	Rha	Fr	H	OMe	OH	H
C30	salsaside F or isomer	Ac	Rha	H	Cm	OH	OH	H
C31	isocistanoside D							
C32	cistanoside K or cistanosinenside A or salsaside E or isomer	Ac	Rha	H/Cf/Cf	Cf/H/H	OMe/OH/OMe	OH/OMe/OH	H

Cf: *trans*-caffeoyl; Cm: *trans*-coumaroyl; Fr: *trans*-feruloyl; Glc: β -glucopyranose; Rha: α -L-rhamnopyranose; Ac: acetyl.

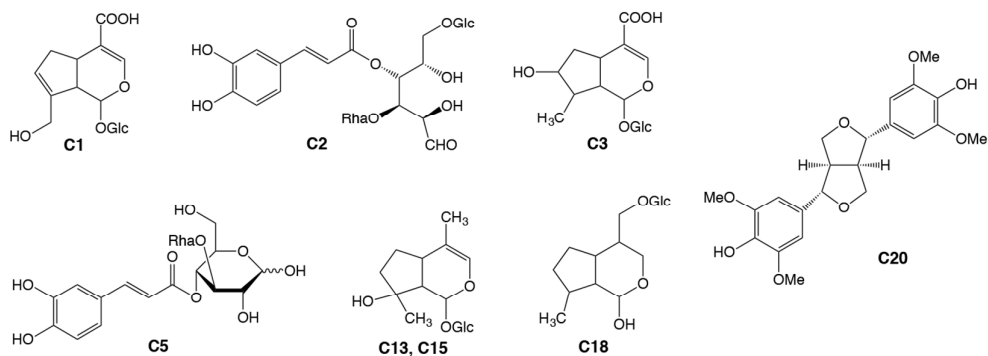
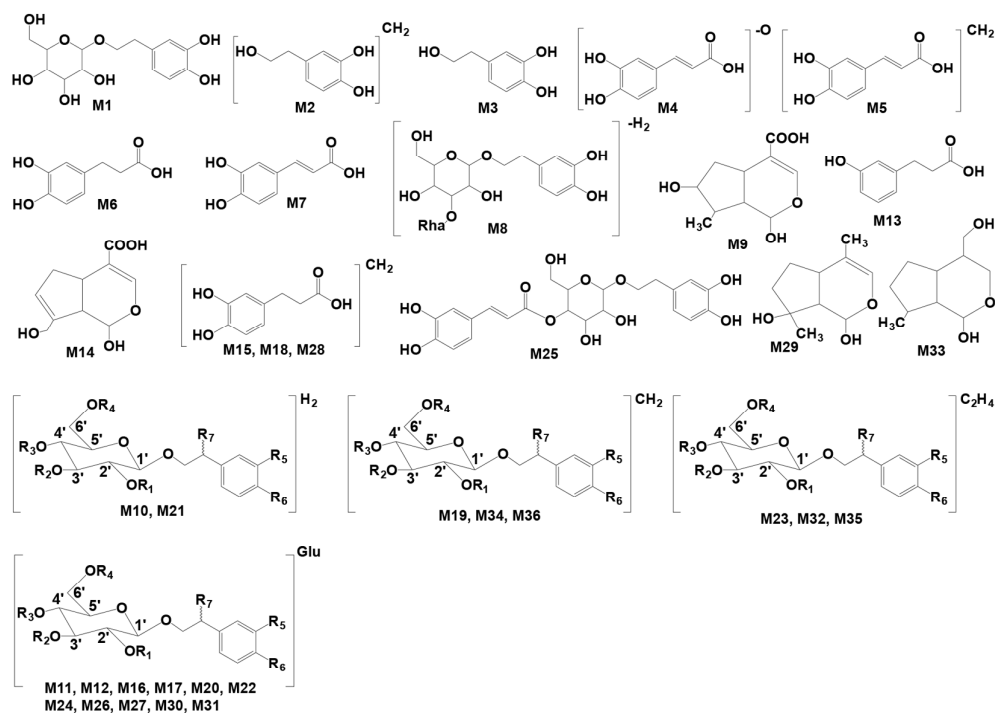


Figure S1



No.	Identification	R1	R2	R3	R4	R5	R6	R7
M10	reduction of cistanbuloside C ₁ /C ₂	H	Rha	Cf	Glc	OH	OH	OH
M11	echinacoside glucuronide conjugation	H	Rha	Cf	Glc	OH	OH	H
M12, 16, 20	syngalide A-3'-α-L-rhamnopyranoside or isomer glucuronide conjugation	H	Rha	Cm/Cf	H	OH/H	OH	H
M17, 22, 27	cistanoside C or isocistanoside C glucuronide conjugation	H	Rha	Cf/H	H/Cf	OMe	OH	H
M19	methylated cistanoside H	Ac	Rha	H	H	OH	OH	H
M21	reduction of echinacoside	H	Rha	Cf	Glc	OH	OH	H
M23	dimethylated echinacoside	H	Rha	Cf	Glc	OH	OH	H
M24	cistanoside E glucuronide conjugation	H	Rha	H	H	OMe	OH	H
M26, 30	2'-acetylacteoside or tubuloside B glucuronide conjugation	Ac	Rha	Cf/H	H/Cf	OH	OH	H
M31	cistanoside D or isocistanoside D glucuronide conjugation	H	Rha	Fr	H	OMe	OH	H
M32, 35	dimethylated acteoside	H	Rha	Cf	H	OH	OH	H
M34, 36	methylated 2'-acetylacteoside or tubuloside B	Ac	Rha	Cf/H	H/Cf	OH	OH	H

Cf: *trans*-caffeoyl; Cm: *trans*-coumaroyl; Fr: *trans*-feruloyl; Glc: β-glucopyranose; Rha: α-L-rhamnopyranose; Ac: acetyl.

Figure S2

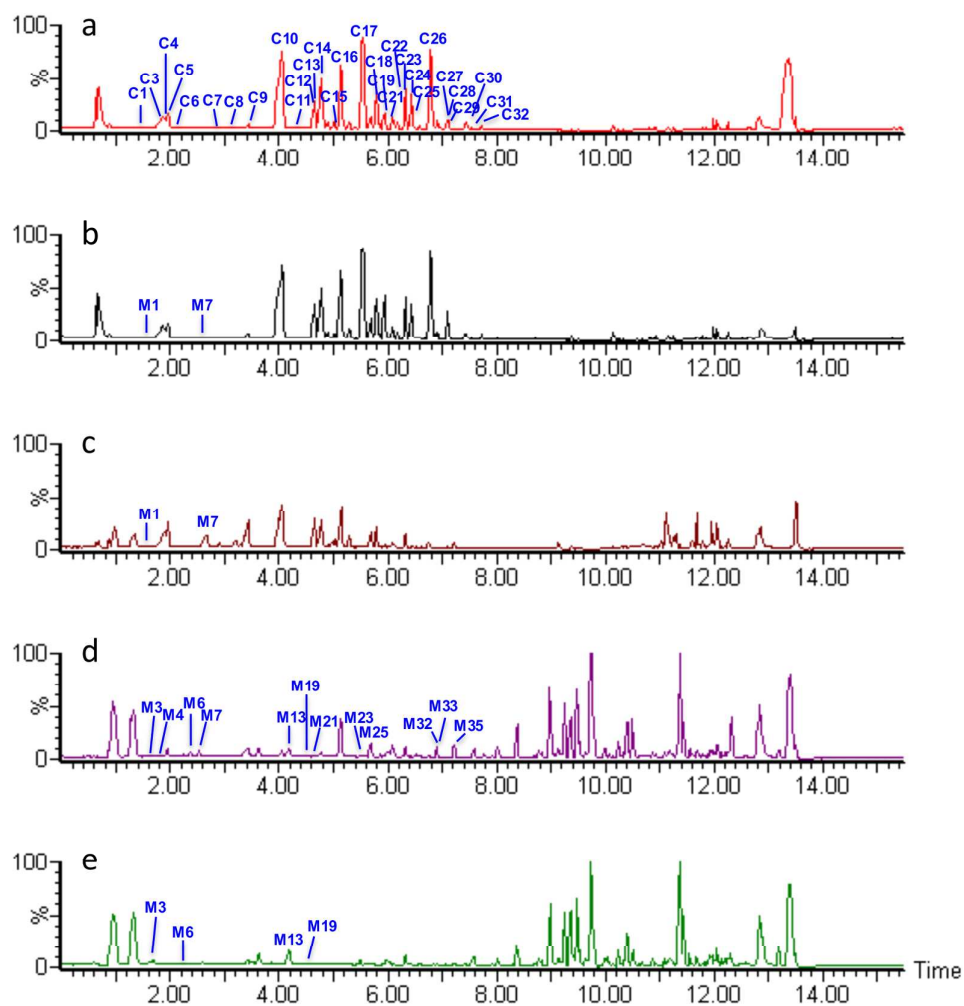


Figure S3