

Supplementary Information

Identification of Hypolipidemic Constituents from *Microctis folium* Using an Integrated Strategy of Chemical Profiling, Pharmacokinetics, Network Pharmacology and Experimental Validation

Zhihao Zeng ^{1,2}, Yanchang Liu ³, Xiaoli Bi ^{1,2}, Wanchun Chen ³, Yunhui Ouyang ³, Jingnian Zhang ^{1,2}, Weitao Chen ^{1,2} and Guanlin Xiao ^{1,2,*}

1 Guangdong Provincial Engineering Technology Research Institute of Traditional Chinese Medicine, Guangzhou 510095, China; zengzhihao129@126.com (Z.Z.); zyfyxjs@gzucm.edu.cn (X.B.); zjn9110@163.com (J.Z.); chenwt009@126.com (W.C.)

2 Guangdong Provincial Key Laboratory of Research and Development in Traditional Chinese Medicine, Guangzhou 510095, China

3 The Fifth Clinical College of Guangzhou University of Chinese Medicine, Guangzhou 510095, China; 1022905399@qq.com (Y.L.); zxzxs202012@163.com (W.C.); 2907228835@qq.com (Y.O.)

* Correspondence: 164669079@qq.com

Table S1 Information on the reference standards

No.	Reference substances	Lot number	Purity	Supplier company
1	Schaftoside	111912-201703	95.6%	National Institutes for Food and Drug Control
2	Berberine	110713-202515	85.9%	National Institutes for Food and Drug Control
3	Isorhamnetin 3- <i>O</i> -neohesperidoside	111571-201205	93.2%	National Institutes for Food and Drug Control
4	Narcissoside	111997-201501	93.1%	National Institutes for Food and Drug Control
5	Caffeic acid	110885-201703	99.7%	National Institutes for Food and Drug Control
6	Epicatechin	110878-201703	99.7%	National Institutes for Food and Drug Control
7	Ferulic acid	110773-201915	99.4%	National Institutes for Food and Drug Control
8	Isoferulin acid	111698-201904	99.3%	National Institutes for Food and Drug Control
9	Rutin	100080-202012	92.2%	National Institutes for Food and Drug Control
10	Vitexin	M-023-120429	>98.0%	Chengdu Herbpurify Co. Ltd.
11	Isovitexin	Y-116-120630	>98.0%	Chengdu Herbpurify Co. Ltd.
12	Typhaneoside	X-020-120305	>98.0%	Chengdu Herbpurify Co. Ltd.
13	<i>N</i> -trans-feruloyltyramine	RFS-F03302108016	>98.0%	Chengdu Herbpurify Co. Ltd.
14	Tiliroside	PRF9101501	>98.0%	Chengdu Herbpurify Co. Ltd.
15	Kaempferol-3- β - <i>O</i> -glucuronide	DSTDS017501	>98.0%	Chengdu Biopurify Phytochemicals Ltd.
16	Sulfamethoxazole	DSTD200226-220	>98.0%	Chengdu Biopurify Phytochemicals Ltd.
17	Cis-p-coumaric acid	AF20041951	>98.0%	Chengdu Alfa Biotechnology Co. Ltd.
18	Violanthin	AF802612	>98.0%	Chengdu Alfa Biotechnology Co. Ltd.
19	<i>N</i> -p-trans-coumaroyltyramine	AF22032709	>98.0%	Chengdu Alfa Biotechnology Co. Ltd.

Table S2. Information on *M. folium*

No.	Batch No.	Collection source	Collection date
S1	20230115	Yangjiang, Guangdong, China	January 2023
S2	20230118	Yangjiang, Guangdong, China	January 2023
S3	20230122	Yangchun, Guangdong, China	January 2023
S4	20230216	Maoming, Guangdong, China	February 2023
S5	20230220	Maoming, Guangdong, China	February 2023
S6	20230318	Huazhou, Guangdong, China	March 2023
S7	20230325	Dianbai, Guangdong, China	March 2023
S8	20230419	Zhanjiang, Guangdong, China	April 2023
S9	20230423	Zhanjiang, Guangdong, China	April 2023
S10	20230514	Maoming, Guangdong, China	May 2023
S11	20230518	Jiangmen, Guangdong, China	May 2023
S12	20230716	Taishan, Guangdong, China	July 2023
S13	20230815	Zhongshan, Guangdong, China	August 2023
S14	20230821	Guangzhou, Guangdong, China	August 2023
S15	20231117	Zhaoqing, Guangdong, China	November 2023
S16	20231123	Yunfu, Guangdong, China	November 2023
S17	20240113	Huizhou, Guangdong, China	January 2024
S18	20240127	Taishan, Guangdong, China	January 2024
S19	20240217	Taishan, Guangdong, China	February 2024
S20	20240228	Zhanjiang, Guangdong, China	February 2024
S21	20240316	Zhanjiang, Guangdong, China	March 2024

Table S3. Parameters of mass spectrometry of the 18 analytes.

No.	Compound	ESI mode	Precursor Ion (m/z)	Product Ion (m/z)	CE (V)
1	Berberine	ESI+	336.1	292.1	36
				321.1	24
2	Caffeic acid	ESI-	179.0	135.0	13
				134.0	13
3	Cis-p-coumaric acid	ESI-	163.0	119.1	16
				92.9	36
4	Epicatechin	ESI-	289.1	245.1	15
				109.0	25
5	Ferulic acid	ESI-	193.1	177.8	12
				134.2	16

6	Isoferulin acid	ESI-	461.0	178.1	8
				134.1	16
7	Isorhamnetin-3- <i>O</i> -neohesperidoside	ESI-	623.2	314.0	35
				271.0	60
8	Isovitexin	ESI-	431.1	311.0	24
				283.1	38
9	Kaempfenol-3- β - <i>O</i> -glucuronide	ESI-	461.0	285.0	20
				228.0	44
10	Narcissoside	ESI-	623.1	314.9	32
				313.9	40
11	<i>N</i> - <i>p</i> -trans-coumaroyltyramine	ESI+	314.1	177.1	16
				145.1	28
12	<i>N</i> -trans-feruloyltyramine	ESI+	284.1	147.1	16
				121.2	28
13	Rutin	ESI-	609.0	301.1	32
				300.1	40
14	Schaftoside	ESI-	563.1	443.2	32
				382.9	40
15	Tiliroside	ESI-	592.9	285.1	32
				284.0	38
16	Typhaneoside	ESI-	769.2	314.0	45
				299.0	65
17	Violanthin	ESI-	577.1	382.9	40
				353.1	40
18	Vitexin	ESI-	431.1	311.1	24
				283.0	36

Table S4 Parameters of mass spectrometry of the eight analytes and IS.

No.	Compound	ESI mode	Precursor Ion (<i>m/z</i>)	Product Ion (<i>m/z</i>)	CE (V)
1	Cis- <i>p</i> -coumaric acid	ESI-	163.0	119.1	16
				92.9	36
2	Ferulic acid	ESI-	193.1	177.8	12
				134.2	16
3	Isoferulin acid	ESI-	461.0	178.1	8
				134.1	16
4	Isovitexin	ESI-	431.1	311.0	24
				283.1	38
5	Kaempfenol-3- β - <i>O</i> -glucuronide	ESI-	461.0	285.0	20
				228.0	44

6	<i>N</i> -p-trans-coumaroyltyramine	ESI+	314.1	177.1	16
				145.1	28
7	<i>N</i> -trans-feruloyltyramine	ESI+	284.1	147.1	16
				121.2	28
8	Vitexin	ESI-	431.1	311.1	24
				283.0	36
9	Sulfamethoxazole (IS)	ESI-	251.9	156.0	12

Table S5 Similarity evaluation of *M. folium* fingerprints for 21 batches.

S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15	S16	S17	S18	S19	S20	S21	Reference
1.000	0.940	0.971	0.950	0.947	0.976	0.944	0.925	0.855	0.925	0.980	0.989	0.965	0.927	0.913	0.982	0.974	0.967	0.876	0.799	0.983	0.983
0.940	1.000	0.938	0.902	0.916	0.963	0.957	0.896	0.931	0.923	0.951	0.949	0.934	0.885	0.919	0.958	0.928	0.936	0.858	0.805	0.979	0.963
0.971	0.938	1.000	0.928	0.936	0.984	0.985	0.873	0.909	0.889	0.962	0.975	0.999	0.88	0.919	0.947	0.988	0.991	0.934	0.869	0.957	0.980
0.950	0.902	0.928	1.000	0.967	0.943	0.925	0.790	0.826	0.785	0.885	0.969	0.93	0.783	0.879	0.917	0.941	0.945	0.779	0.665	0.937	0.907
0.947	0.916	0.936	0.967	1.000	0.933	0.928	0.785	0.832	0.802	0.908	0.978	0.942	0.779	0.900	0.921	0.954	0.931	0.804	0.681	0.943	0.915
0.976	0.963	0.984	0.943	0.933	1.000	0.982	0.887	0.940	0.904	0.957	0.978	0.977	0.891	0.953	0.965	0.971	0.993	0.895	0.838	0.981	0.983
0.944	0.957	0.985	0.925	0.928	0.982	1.000	0.842	0.942	0.865	0.937	0.958	0.986	0.841	0.925	0.929	0.973	0.986	0.916	0.857	0.949	0.965
0.925	0.896	0.873	0.790	0.785	0.887	0.842	1.000	0.772	0.991	0.961	0.871	0.857	0.997	0.792	0.952	0.873	0.859	0.863	0.847	0.919	0.947
0.855	0.931	0.909	0.826	0.832	0.94	0.942	0.772	1.000	0.825	0.857	0.879	0.901	0.778	0.950	0.859	0.866	0.924	0.832	0.814	0.908	0.902
0.925	0.923	0.889	0.785	0.802	0.904	0.865	0.991	0.825	1.000	0.969	0.882	0.874	0.990	0.835	0.957	0.881	0.873	0.881	0.869	0.936	0.959
0.980	0.951	0.962	0.885	0.908	0.957	0.937	0.961	0.857	0.969	1.000	0.962	0.955	0.961	0.889	0.978	0.959	0.942	0.909	0.856	0.973	0.990
0.989	0.949	0.975	0.969	0.978	0.978	0.958	0.871	0.879	0.882	0.962	1.000	0.973	0.871	0.936	0.961	0.973	0.970	0.852	0.760	0.983	0.968
0.965	0.934	0.999	0.93	0.942	0.977	0.986	0.857	0.901	0.874	0.955	0.973	1.000	0.863	0.910	0.939	0.989	0.987	0.932	0.861	0.950	0.973
0.927	0.885	0.88	0.783	0.779	0.891	0.841	0.997	0.778	0.990	0.961	0.871	0.863	1.000	0.800	0.950	0.875	0.866	0.873	0.861	0.916	0.949
0.913	0.919	0.919	0.879	0.900	0.953	0.925	0.792	0.95	0.835	0.889	0.936	0.910	0.800	1.000	0.890	0.891	0.932	0.785	0.733	0.944	0.914
0.982	0.958	0.947	0.917	0.921	0.965	0.929	0.952	0.859	0.957	0.978	0.961	0.939	0.950	0.890	1.000	0.959	0.948	0.893	0.832	0.981	0.985
0.974	0.928	0.988	0.941	0.954	0.971	0.973	0.873	0.866	0.881	0.959	0.973	0.989	0.875	0.891	0.959	1.000	0.983	0.932	0.851	0.949	0.974
0.967	0.936	0.991	0.945	0.931	0.993	0.986	0.859	0.924	0.873	0.942	0.97	0.987	0.866	0.932	0.948	0.983	1.000	0.916	0.853	0.957	0.973
0.876	0.858	0.934	0.779	0.804	0.895	0.916	0.863	0.832	0.881	0.909	0.852	0.932	0.873	0.785	0.893	0.932	0.916	1.000	0.977	0.854	0.933
0.799	0.805	0.869	0.665	0.681	0.838	0.857	0.847	0.814	0.869	0.856	0.760	0.861	0.861	0.733	0.832	0.851	0.853	0.977	1.000	0.788	0.885
0.983	0.979	0.957	0.937	0.943	0.981	0.949	0.919	0.908	0.936	0.973	0.983	0.950	0.916	0.944	0.981	0.949	0.957	0.854	0.788	1.000	0.981

Table S6 Linear ranges, regression equations, correlation coefficients, quantification limits of 18 components.

No.	Compounds	Regression equation	Range (ng/mL)	R	LLOQ (ng/mL)
1	Berberine	$Y=3718.410X+992.170$	0.012~121.210	0.998	0.024
2	Caffeic acid	$Y=92.488X+3196.989$	2.068~2068.000	0.999	0.041
3	Cis-p-coumaric acid	$Y=67.807X+28607.532$	1.000~1000.000	0.998	0.020
4	Epicatechin	$Y=22.407X+6276.769$	111.200~35584.000	0.999	0.111
5	Ferulic acid	$Y=21.016X+1308.772$	104.580~1673.280	1.000	0.205
6	Isoferulin acid	$Y=1.185X-415.286$	322.400~41267.200	0.999	0.322
7	Isorhamnetin-3-O-neohesperidoside	$Y=63.793X+13493.205$	65.706~8410.368	0.999	0.066
8	Isovitexin	$Y=81.336X+14007.920$	252.350~16150.400	0.999	0.504
9	Kaempfenol-3-β-O-glucuronide	$Y=43.706X+485.329$	0.012~124.460	0.998	0.024
10	Narcissoside	$Y=7.995X+1590.879$	122.659~15700.380	0.999	0.245
11	N-p-trans-coumaroyltyramine	$Y=1077.021X+577308.143$	1.465~146.510	0.996	0.293
12	N-trans-feruloyltyramine	$Y=1420.386X+538000.889$	0.310~150.500	0.997	0.062
13	Rutin	$Y=45.748X-8125.029$	124.500~7968.000	0.999	0.125
14	Schaftoside	$Y=23.173X+3905.399$	2.928~292.755	0.999	0.293
15	Tiliroside	$Y=48.708X+9904.386$	129.850~2077.600	0.995	0.026
16	Typhaneoside	$Y=25.304X+3559.870$	0.786~392.980	1.000	0.157
17	Violanthin	$Y=24.969X+3659.782$	125.000~16000.000	1.000	0.250
18	Vitexin	$Y=95.438X-238.899$	185.000~23680.000	1.000	0.185

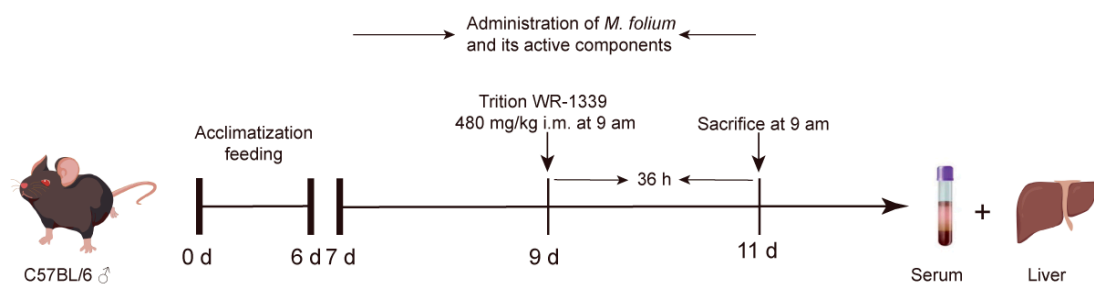
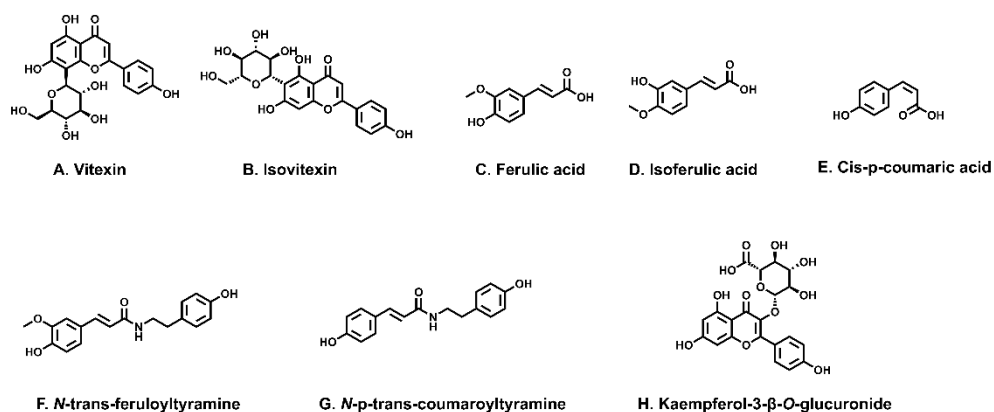
Table S7 Quantitative analysis by UPLC-QQQ-MS/MS for 21 batches *M. folium* ($n = 2$).

Compounds	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15	S16	S17	S18	S19	S20	S21	
Berberine (μg/g)	0.154	0.147	0.069	0.107	0.077	0.031	0.163	0.049	0.028	0.358	0.033	0.065	0.018	0.016	0.105	0.081	0.020	0.016	0.018	0.016	0.026	
Caffeic acid (μg/g)	39.119	20.450	21.054	32.470	25.959	23.351	7.470	87.447	18.590	68.190	51.499	30.412	14.523	55.973	102.16	1.756	17.061	33.665	16.318	59.167	21.265	
Cis-p-coumaric acid (μg/g)	7																					
	20.593	55.005	31.832	43.126	6.912	29.569	38.980	88.884	90.933	68.283	33.926	0.305	12.209	73.926	4.453	25.782	7.578	79.230	42.919	127.39	15.511	
	3																					
Epicatechin (μg/g)	1221.2	377.08	342.94	839.18	390.56	524.79	166.32	881.37	135.90	784.92	1564.1	1154.1	341.50	551.98	489.05	291.73	150.85	1069.5	1057.6	264.74	2756.9	
	15	8	0	2	5	8	6	7	1	9	58	20	0	7	7	7	7	11	57	0	80	
Ferulic acid (μg/g)	119.25	70.507	77.694	93.223	73.906	90.914	80.598	144.18	107.95	138.51	124.90	100.47	59.263	128.18	154.60	75.306	58.636	156.42	83.348	113.55	93.675	
	4							9	2	4	8	3		0	3			8		2		
Isoferulin acid (μg/g)	253.95	1385.4	1532.7	167.91	239.44	125.31	184.88	212.11	2063.6	2625.2	171.58	216.12	96.000	2391.3	208.24	154.55	107.18	237.25	214.38	161.80	190.72	
	8	19	08	8	2	9	5	8	12	49	6	0		55	5	6	0	4	4	3	0	
Isorhamnetin-3-O-neohesperid	561.14	304.46	13.182	10.072	77.426	0.324	333.37	625.83	263.26	537.79	564.23	57.296	383.79	587.89	348.55	61.683	304.22	491.17	450.00	419.82	441.03	
oside (μg/g)	2	0						6	9	1	0	2		7	6	0		1	0	0	6	4
Isovitexin (μg/g)	1088.4	486.62	540.13	354.72	288.35	485.64	322.64	2349.9	353.87	2237.5	1462.2	745.07	367.16	2194.4	576.86	692.07	268.36	609.04	889.34	1237.8	877.73	
	53	3	2	5	1	7	4	58	0	36	61	3	6	29	3	4	5	6	5	70	3	
Kaempfenol-3-β-O-glucuronid	0.003	0.002	0.003	0.002	0.002	0.002	0.003	0.033	0.005	0.004	0.002	0.012	0.253	0.004	0.005	0.001	0.001	0.579	0.001	0.001	0.002	
e (μg/g)																						
Narcissoside (μg/g)	10129.	5797.9	8149.7	8933.8	7649.3	6436.1	6368.5	11375.	5179.6	9928.8	10373.	9527.8	7129.6	10748.	6549.2	6667.8	5788.1	9168.3	8364.5	8189.0	8204.5	
	298	09	49	55	97	39	21	431	42	14	353	48	17	127	20	63	15	82	55	30	40	
N-p-trans-coumaroyltyramine	3.094	2.489	2.613	2.241	5.787	3.690	3.119	1.964	1.514	2.255	1.880	4.681	3.551	1.975	4.647	4.391	3.736	2.172	2.158	1.742	2.650	
(μg/g)																						
N-trans-feruloyltyramine (μg/g)	0.457	0.471	0.736	0.582	1.206	0.813	0.639	0.156	0.278	0.193	0.344	0.829	0.819	0.230	0.651	1.530	0.913	0.402	0.183	0.084	0.280	
Rutin (μg/g)	579.55	113.72	162.62	378.04	700.69	183.76	406.63	510.27	79.758	456.19	452.33	550.49	147.57	521.60	535.95	115.22	240.62	215.76	435.42	122.02	210.74	
	8	1	3	1	3	8	5	3		5	7	5	5	7	5	2	5	9	4	9	2	

Schaftoside (µg/g)	6.558	5.137	7.199	3.873	3.455	4.211	4.468	15.308	6.813	13.522	7.142	5.363	6.519	12.307	4.002	5.781	3.620	7.428	19.424	19.221	6.436
Tiliroside (µg/g)	51.348	76.691	72.094	47.287	30.295	43.473	8.887	162.55	56.544	177.64	104.53	47.604	33.322	162.06	63.504	130.91	53.392	79.743	201.98	324.14	67.444
								9		9	0			7		5			3	8	
Typhaneoside (µg/g)	34.984	5.037	229.18	234.69	316.32	86.630	1.529	0.209	0.018	0.023	0.013	381.42	27.606	7.111	28.872	203.18	38.709	6.924	5.902	40.551	140.59
			8	3	4							3				5				8	
Violanthin (µg/g)	1916.4	1119.6	2132.1	1150.4	955.49	1431.0	1591.2	2909.3	2199.6	3002.9	2600.1	1705.6	1859.1	2558.0	1468.5	1112.9	1210.7	2339.1	3822.0	4583.2	1422.1
	84	55	57	42	0	87	94	03	78	74	37	26	84	80	73	64	81	95	34	17	27
Vitexin (µg/g)	645.60	291.60	300.20	171.63	178.18	311.33	141.48	1539.7	202.70	1418.2	837.89	408.39	224.43	1446.9	278.40	455.64	175.34	313.92	744.35	1022.2	526.73
	3	8	6	9	1	1	3	59	6	20	6	3	8	07	3	3	4	0	8	46	0

Table S8 Regression equations, linear ranges, correlation coefficients and LLOQs of analytes.

No.	Component	Calibration Curve	<i>R</i>	Range (ng/mL)	LLOQ (ng/mL)
1	Vitexin	$Y=2.038584X+0.023845$	0.996	0.0231~1480.00	0.0462
2	Isovitexin	$Y=2.661183X+0.010128$	0.998	0.0315~2018.80	0.0630
3	Ferulic acid	$Y=0.82016X+0.006945$	0.997	0.0523~3346.56	0.1046
4	Isoferulic acid	$Y=0.082112X-0.00023$	0.991	0.1209~7737.60	0.2418
5	Cis-p-coumaric acid	$Y=3.428359X+0.173394$	0.994	0.0311~2000.00	0.0622
6	Kaempferol-3- β -O-glucuronide	$Y=1.236311X+0.002697$	0.991	0.0311~1991.30	0.0622
7	<i>N</i> -trans-feruloyltyramine	$Y=1.27156X+1.042404$	0.992	0.0011~60.20	0.0022
8	<i>N</i> -p-trans-coumaroyltyramine	$Y=2.22626X+2.05953$	0.997	0.0092~586.00	0.0184

**Figure S1** Process of administration of *M. folium* and its active components and hyperlipemia modeling.**Figure S2** The structural formulas of eight compounds.