

Supporting Information

Induction of apoptosis via inactivating PI3K/AKT pathway in colorectal cancer cells using aged Chinese Hakka stir-fried green tea extract

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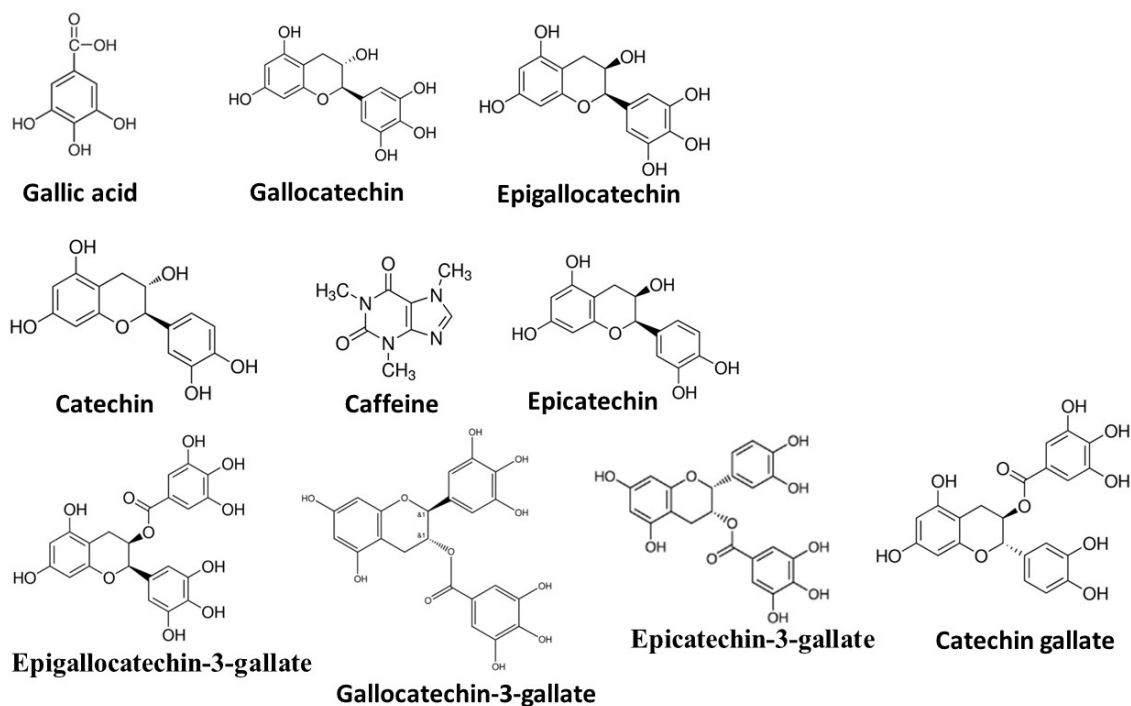
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(A) HPLC analysis of standard compounds for the verification of the extracted compounds from aged Chinese green tea (HSGT)

A list of standard compounds purchased from commercial:

Gallic acid: **GA**; Gallocatechin: **GC**; Epigallocatechin: **EGC**; Catechin: **C**; Caffeine: **CAFF**; Epicatechin: **EC**; Epigallocatechin-3-gallate: **EGCG**; Gallocatechin-3-gallate: **GCG**; Epicatechin-3-gallate: **ECG**; Catechin gallate: **CG**



HPLC analytical results for a mixture of the standards:

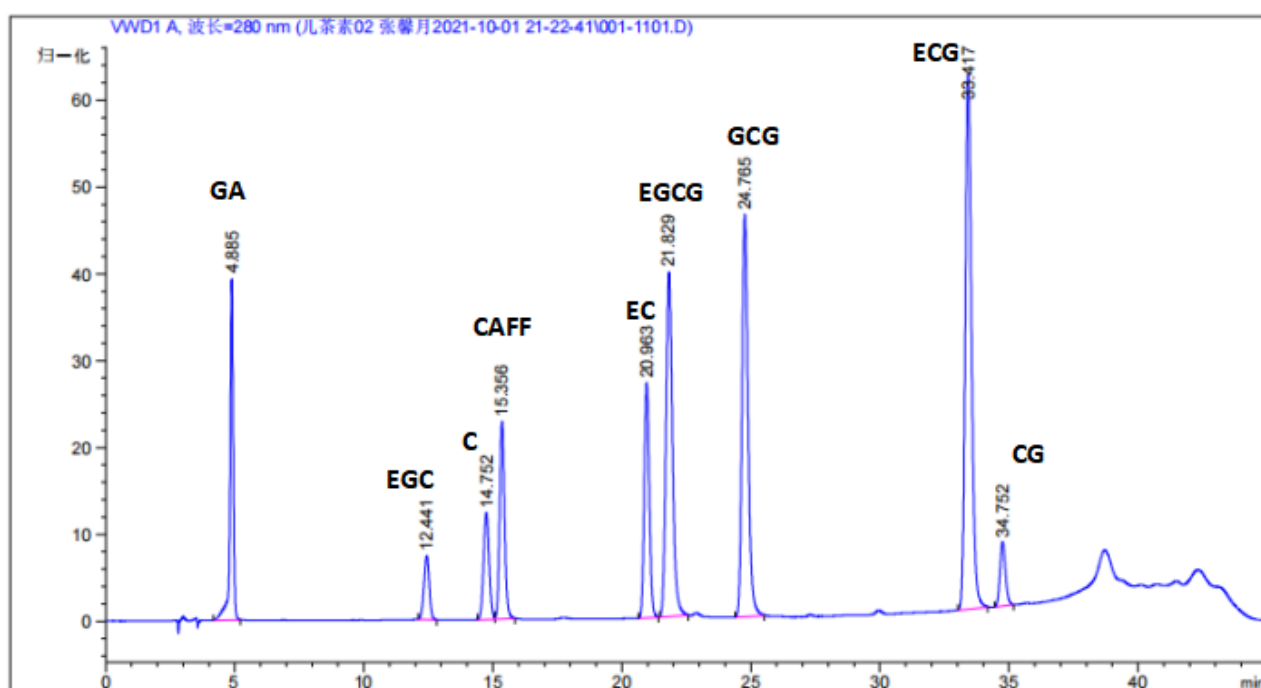


Figure S1: A HPLC chromatogram of a mixture of the standard compounds (HPLC detection wavelength: 280 nm). The compound corresponding to its retention time is labelled and summarized in the Table below.

Standard compound	Retention time (min)
GA	4.885
GC	7.514
EGC	12.441
C	14.752
CAFF	15.356
EC	20.963
EGCG	21.829
GCG	24.765
ECG	33.417
CG	34.752

(B) Qualitative and quantitative analysis of the compounds extracted from aged Chinese green tea (HSGT) with HPLC using external standard method

1. Analysis for the extract from tea sample: 03Y

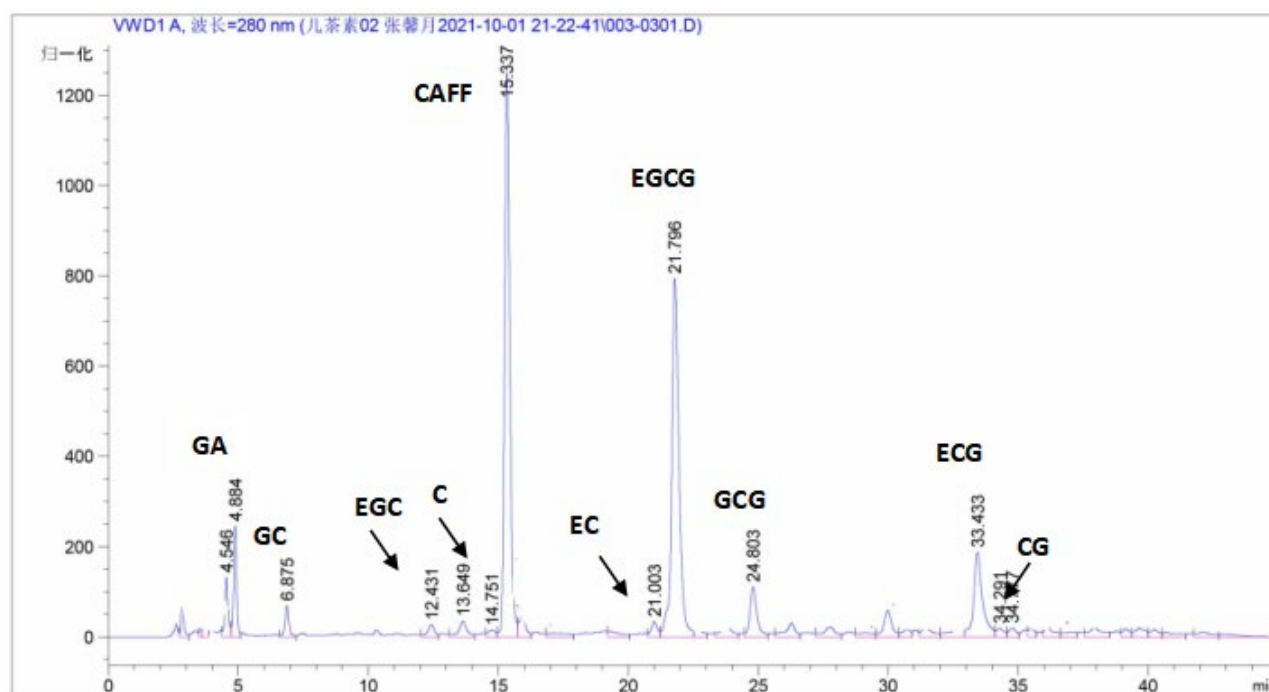


Figure S2: Compounds identified including: CG, catechin gallate; ECG, Epicatechin-3-gallate; GCG, gallocatechin-3-gallate; EGCG, epigallocatechin-3-gallate; EC, epicatechin; C, catechin; GC, galocatechin; EGC, epigallocatechin; GA, gallic acid; CAFF, caffeine.

2. Analysis for the extract from tea sample: 07Y

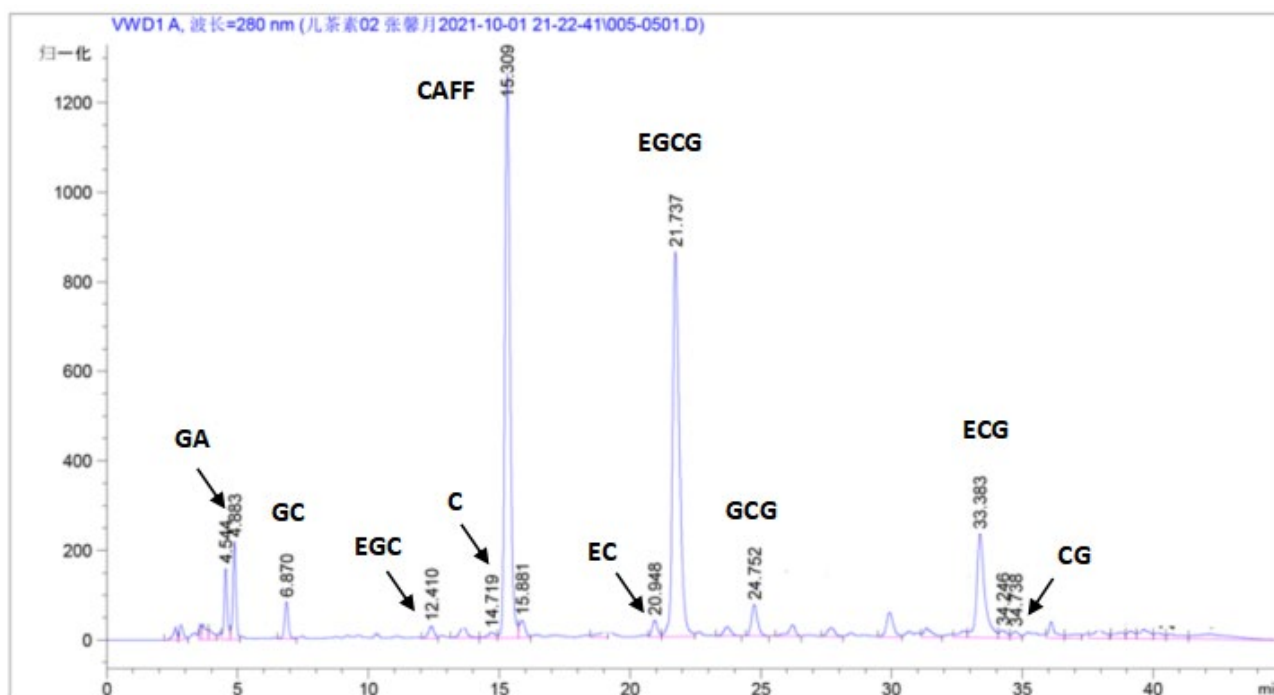


Figure S3: Compounds identified including: CG, catechin gallate; ECG, Epicatechin-3-gallate; GCG, gallocatechin-3-gallate; EGCG, epigallocatechin-3-gallate; EC, epicatechin; C, catechin; GC, gallocatechin; EGC, epigallocatechin; GA, gallic acid; CAFF, caffeine.

3. Analysis for the extract from tea sample: 11Y

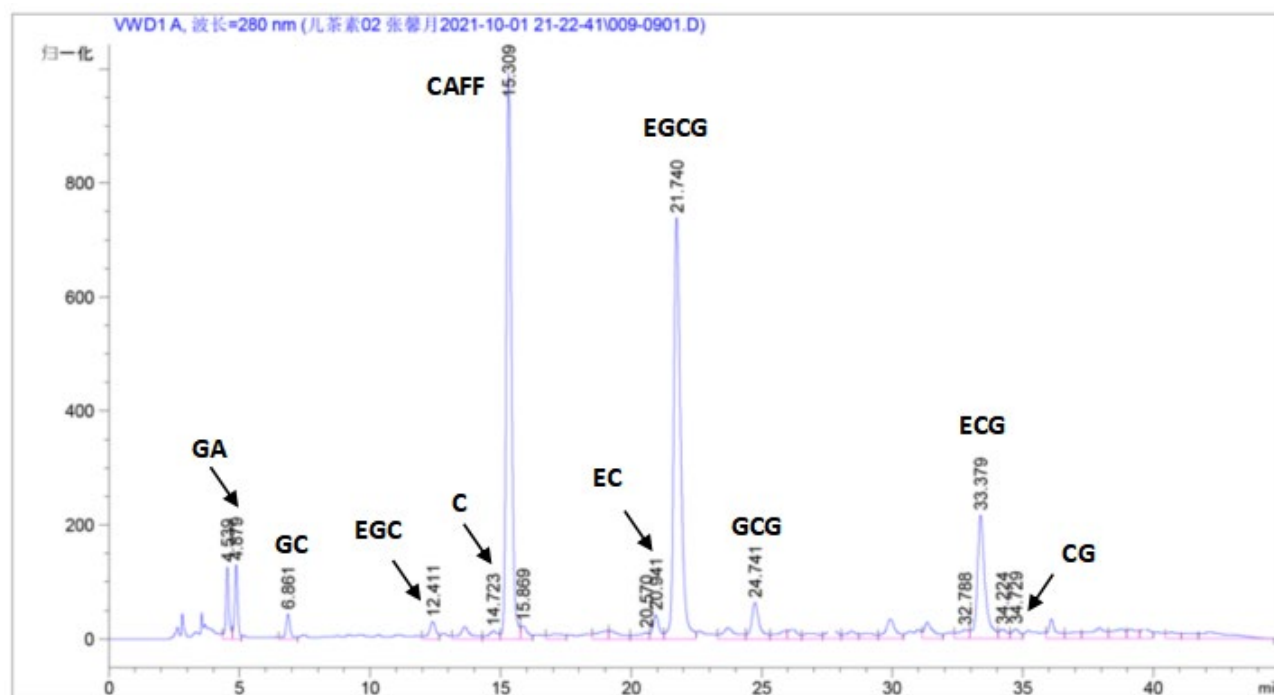


Figure S4: Compounds identified including: CG, catechin gallate; ECG, Epicatechin-3-gallate; GCG, gallocatechin-3-gallate; EGCG, epigallocatechin-3-gallate; EC, epicatechin; C, catechin; GC, gallocatechin; EGC, epigallocatechin; GA, gallic acid; CAFF, caffeine.

4. Analysis for the extract from tea sample: 15Y

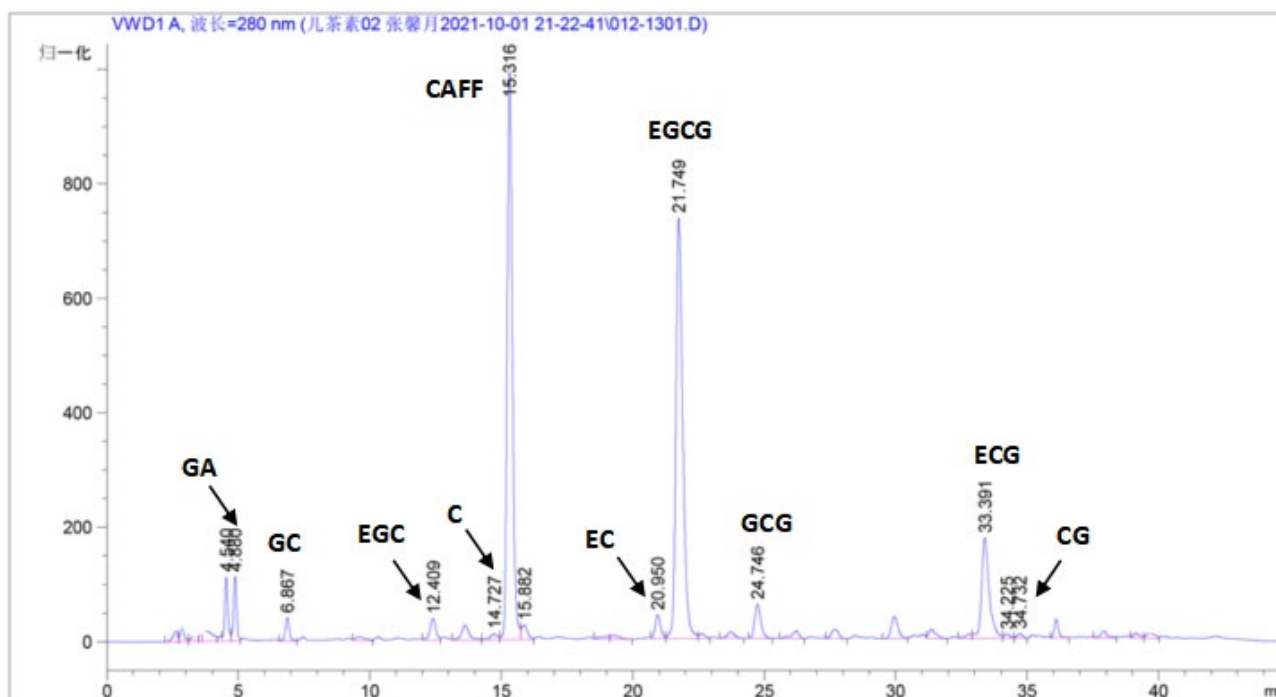


Figure S5: Compounds identified including: CG, catechin gallate; ECG, Epicatechin-3-gallate; GCG, gallocatechin-3-gallate; EGCG, epigallocatechin-3-gallate; EC, epicatechin; C, catechin; GC, gallocatechin; EGC, epigallocatechin; GA, gallic acid; CAFF, caffeine.

5. Analysis for the extract from tea sample: 19Y

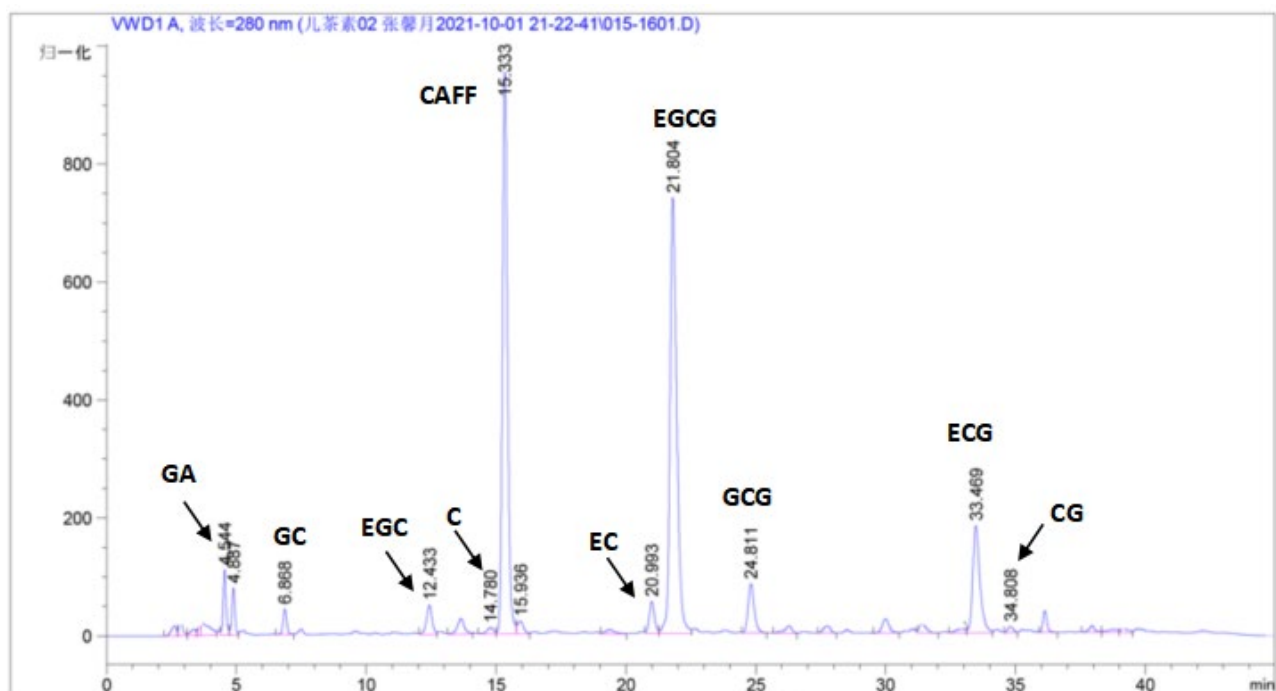


Figure S6: Compounds identified including: CG, catechin gallate; ECG, Epicatechin-3-gallate; GCG, gallocatechin-3-gallate; EGCG, epigallocatechin-3-gallate; EC, epicatechin; C, catechin; GC, gallocatechin; EGC, epigallocatechin; GA, gallic acid; CAFF, caffeine.

Table S1. The compounds identified from the extracts of the aged Chinese green tea (HSGT)

Compound/Peak Area	GA	GC	EGC	C	CAFF	EC	EGCG	GCG	ECG	CG
Retention Time (min)	4.88	7.51	13.05	15.41	16.02	22.38	23.15	26.23	36.00	37.00
Standard mixture 1	338.54	0	104.24	181.22	301.11	367.07	683.22	758.56	1024.84	105.39
Standard mixture 2	337.76	0	104.48	184.31	299.87	369.82	684.33	748.87	1027.66	109.01
03Y-1	1989.29	669.85	430.59	359.61	17552.30	538.93	15592.00	2035.02	4381.74	361.99
03Y-2	2024.96	755.60	498.01	317.40	17457.10	566.68	15413.00	2101.71	4452.00	403.33
03Y-3	1905.78	674.58	373.01	292.38	16406.30	407.76	14168.50	1684.76	3684.00	156.92
07Y-1	1781.31	866.42	412.85	245.23	17363.10	534.38	15654.70	1137.43	4968.73	273.67
07Y-2	1797.10	906.43	402.83	242.58	17489.80	527.47	15451.20	1204.82	4910.65	287.38
07Y-3	1755.44	889.28	435.00	294.38	16887.30	579.49	15153.00	1327.62	4578.22	247.20
11Y-1	1300.96	591.46	597.70	344.70	16503.80	772.28	16537.40	1432.15	5254.98	269.38
11Y-2	1073.88	517.24	547.85	347.89	13690.70	720.56	13506.10	1339.82	4533.44	366.64
11Y-3	1167.52	525.64	536.31	299.88	14789.20	686.25	14853.50	1269.57	4675.50	227.66
15Y-1	1021.42	501.54	681.36	277.41	14719.90	731.77	14915.20	1199.43	3923.16	126.56
15Y-2	937.72	445.50	589.87	216.09	13558.70	614.45	13324.00	966.42	3542.82	115.71
15Y-3	1113.49	551.73	744.71	300.36	16228.70	801.78	16488.20	1347.79	4342.29	141.71
19Y-1	674.92	459.86	794.61	229.22	13440.10	831.37	14164.20	1445.18	3691.57	174.80
19Y-2	652.98	439.22	748.73	212.52	12902.20	780.80	13388.90	1370.18	3486.25	149.92
19Y-3	672.27	499.59	787.00	245.46	13168.70	798.72	14008.40	1667.30	3672.93	193.24

Table S2. The content of each compound determined from the extracts of the aged Chinese green tea (HSGT)

Sample/Content (mg/g)	GA	CAFF	CG	ECG	GCG	EGCG	EC	C	GC	EGC
03Y-1	11.49	100.20	2.59	33.48	18.50	151.15	9.76	7.03	69.02	37.40
03Y-2	10.85	92.42	2.69	31.55	17.72	138.59	9.54	5.77	72.27	39.90
03Y-3	10.91	92.92	1.00	27.91	15.21	136.51	7.24	5.70	69.02	32.37
Average	10.88	92.67	2.64	29.73	16.47	137.55	9.65	5.74	69.02	38.65
SD	0.03	0.25	0.05	0.96	0.39	1.04	0.11	0.03	0.00	1.25
07Y-1	9.20	88.98	1.71	34.11	9.29	140.08	8.69	4.34	80.30	32.25
07Y-2	9.96	96.15	1.94	36.16	10.55	144.26	9.19	4.61	90.13	33.79
07Y-3	9.62	91.91	1.62	33.37	11.51	140.16	10.04	5.51	87.57	36.03
Average	9.79	90.45	1.67	33.74	10.45	141.79	9.31	4.48	86.00	33.02
SD	0.17	1.47	0.04	0.37	0.48	0.04	0.25	0.13	1.28	0.77
11Y-1	6.30	80.69	1.79	38.50	12.47	153.46	13.57	6.46	58.31	49.16
11Y-2	7.05	75.95	2.51	33.14	11.65	151.77	12.62	6.51	50.86	45.12
11Y-3	5.85	75.74	1.51	34.75	11.22	136.25	12.20	5.72	52.55	44.94
Average	6.40	75.85	1.65	33.94	11.44	146.77	12.41	6.49	51.71	45.03
SD	0.22	0.10	0.14	0.81	0.21	0.85	0.21	0.02	0.85	0.09
15Y-1	5.55	81.64	0.75	29.16	10.62	140.85	13.05	5.31	50.19	56.77
15Y-2	5.12	90.05	0.68	26.60	8.65	127.38	11.02	4.21	45.02	49.90
15Y-3	5.94	87.93	0.84	31.51	11.64	125.42	13.99	5.60	53.92	60.41
Average	5.54	88.99	0.76	30.34	11.13	131.21	13.52	5.46	52.05	58.59
SD	0.33	1.06	0.04	1.18	0.51	0.98	0.47	0.14	1.87	1.82
19Y-1	3.45	72.63	1.09	26.76	12.48	130.57	14.52	4.30	44.86	64.36
19Y-2	3.37	70.63	0.92	25.60	12.00	125.19	13.80	4.05	43.41	61.56
19Y-3	3.51	72.61	1.25	27.17	14.70	131.82	14.22	4.70	49.78	65.08
Average	3.45	71.96	1.08	26.51	12.24	131.20	14.37	4.35	44.13	63.67
SD	0.03	0.01	0.08	0.21	0.24	0.63	0.15	0.26	0.73	0.36

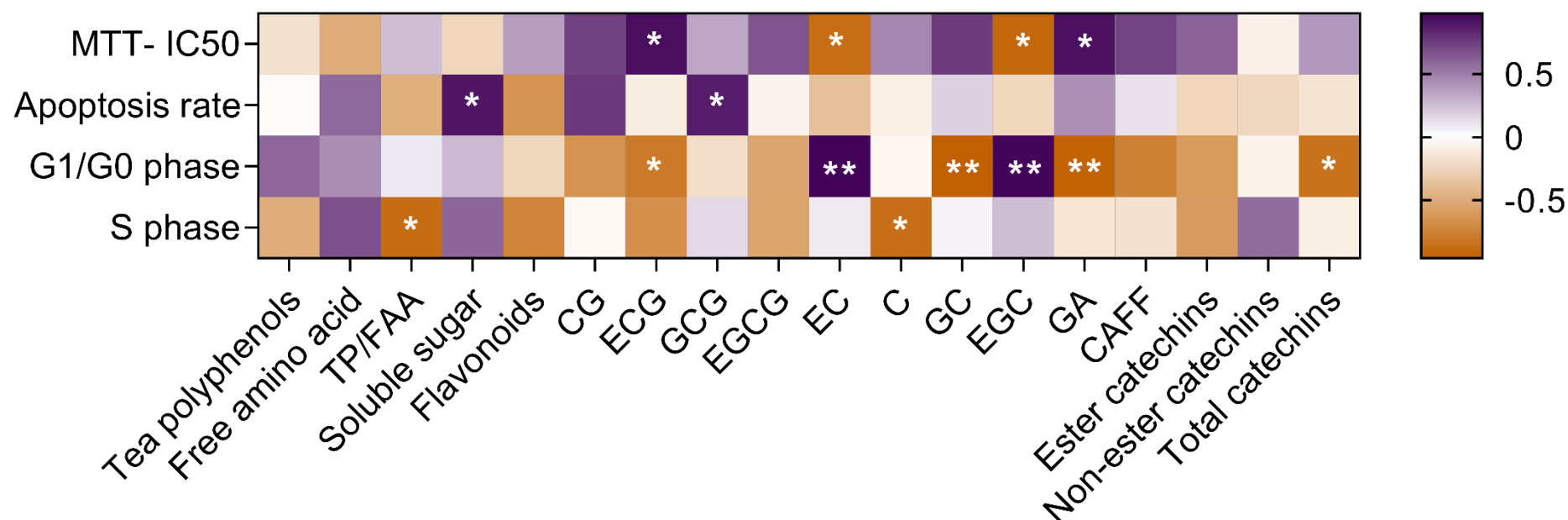


Figure S7. Correlation heatmap analysis of HSGTs extract active ingredients with HT-29 cell-related indexes. The HSGTs extracts were obtained from five different tea samples including **03Y**, **07Y**, **11Y**, **15Y** and **19Y**. The HT-29 cell-related indexes include cell proliferation (MTT-IC₅₀, at 48 h), cell apoptosis (Apoptosis rate, at 48 h 0.2 mg/mL), and cell cycle blockade (G1/G0 phase, S phase, at 48 h 0.2 mg/mL). Pearson correlation analysis was performed. Purple and brown squares indicated positively and negatively correlation, respectively. The *p*-value was calculated from the one-tailed option with a confidence interval of 95%. The statistically significant correlation was showed as **p* < 0.05 and ***p* < 0.01.