Evaluation of the Leaves and Seeds of Cucurbitaceae Plants as a New Source of Bioactive Compounds for Colorectal Cancer Prevention and Treatment

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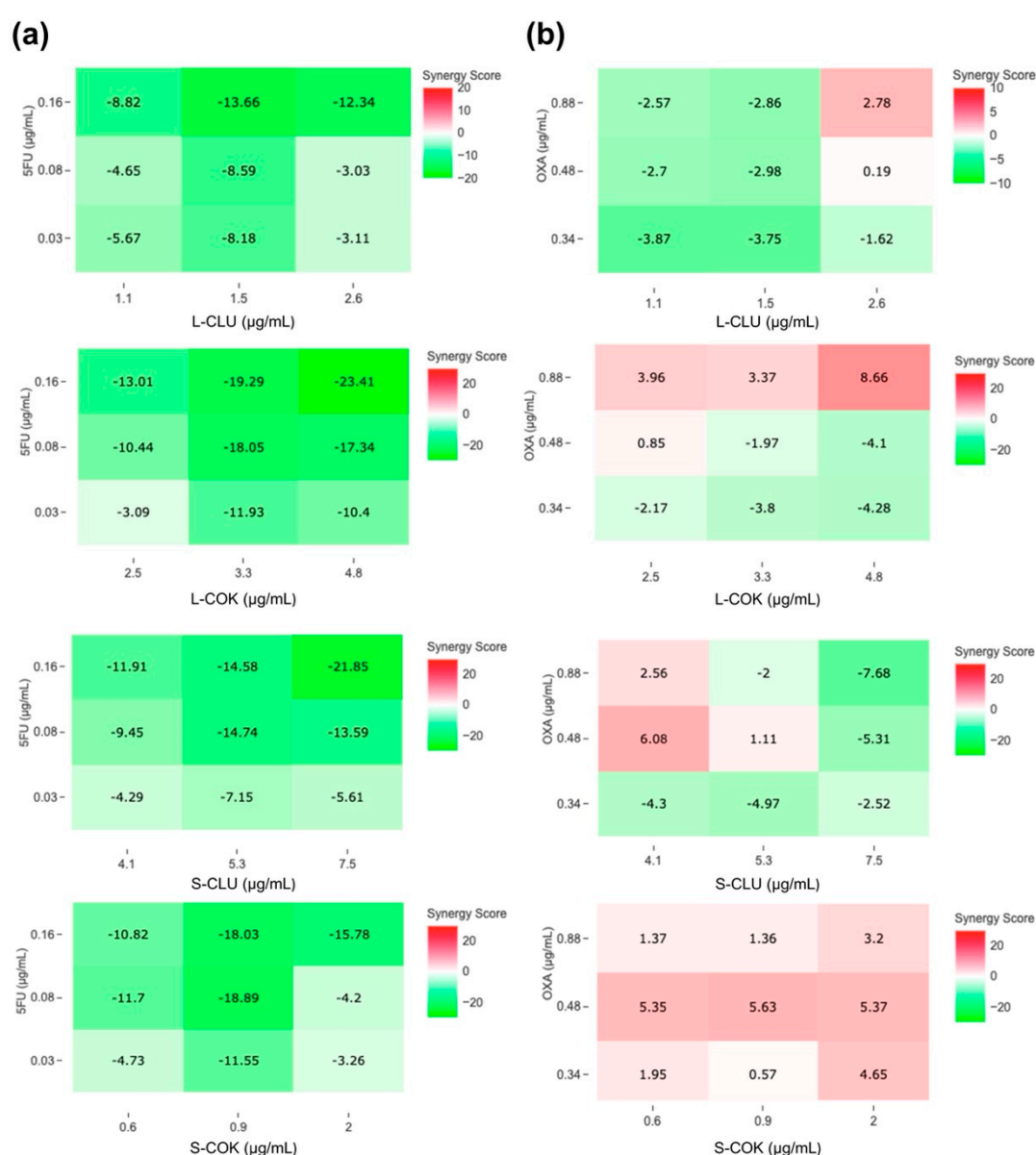
Supplementary material:

Figure S1. Evaluation of synergistic effect of cEtOH extracts from CLU and COK plants with chemotherapy drugs by Bliss model. T84 cells were treated with cEtOH extracts from leaves and

seed of CLU and COK plants in combination with **(a)** 5-FU or **(b)** OXA. Synergy scores calculated by the Bliss model were plotted on heat maps using SynergyFinder Plus, distinguishing areas of synergy in red (values >10), addition in white (from -10 to 10), or antagonism in green (<-10).

Figure S2. Evaluation of synergistic effect of cEtOH extracts from CLU and COK plants with chemotherapy drugs by Loewe model. T84 cells were treated with cEtOH extracts from leaves and seed of CLU and COK plants in combination with **(a)** 5-FU or **(b)** OXA. Synergy scores calculated by the Loewe model were plotted on heat maps using SynergyFinder Plus, distinguishing areas of synergy in red (values >10), addition in white (from -10 to 10), or antagonism in green (<-10).
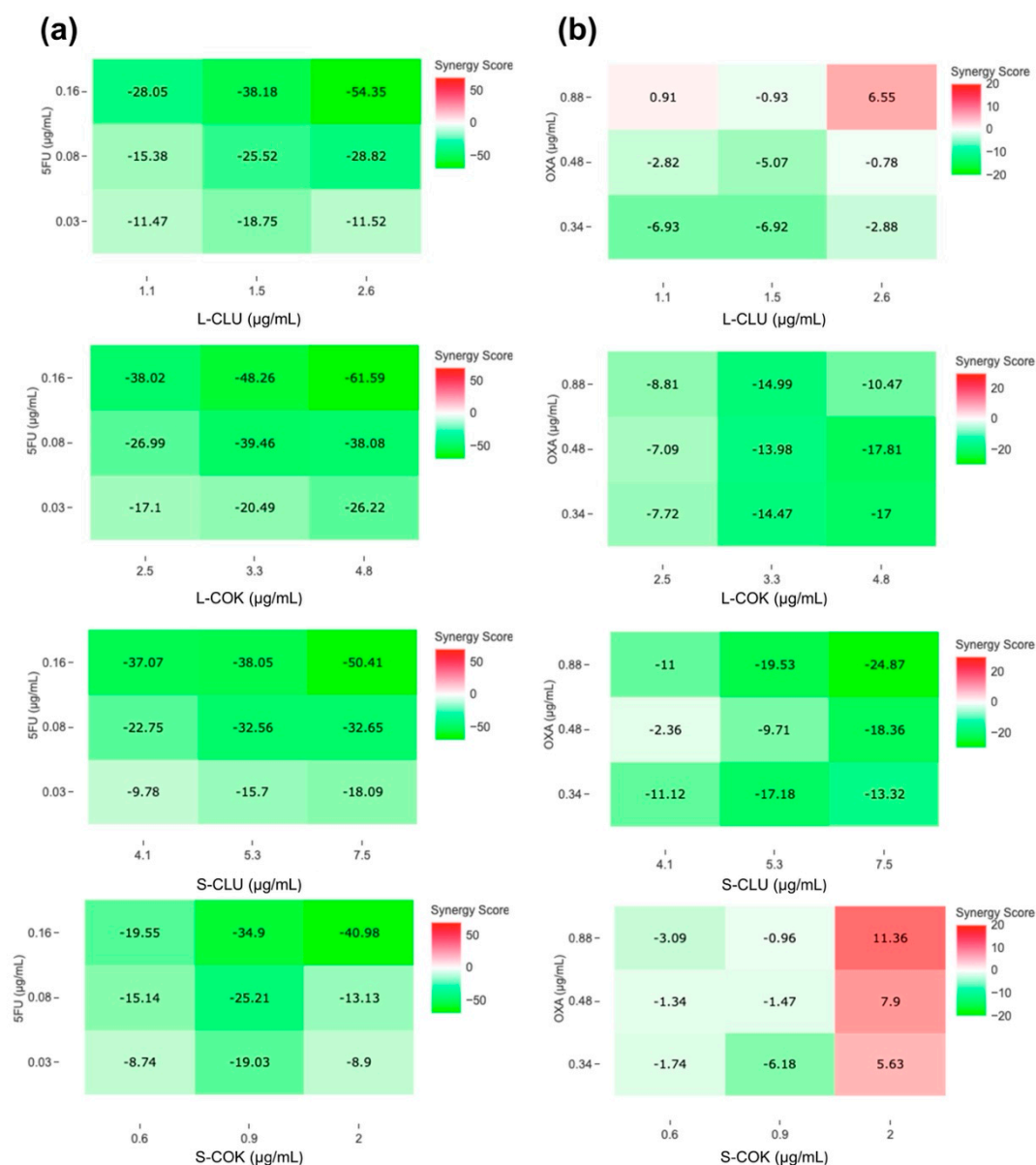
Figure S3. Evaluation of synergistic effect of cEtOH extracts from CLU and COK plants with chemotherapy drugs by ZIP model. T84 cells were treated with cEtOH extracts from leaves and seed of CLU and COK plants in combination with **(a)** 5-FU or **(b)** OXA. Synergy scores calculated by the ZIP model were plotted on heat maps using SynergyFinder Plus, distinguishing areas of synergy in red (values >10), addition in white (from -10 to 10), or antagonism in green (<-10).
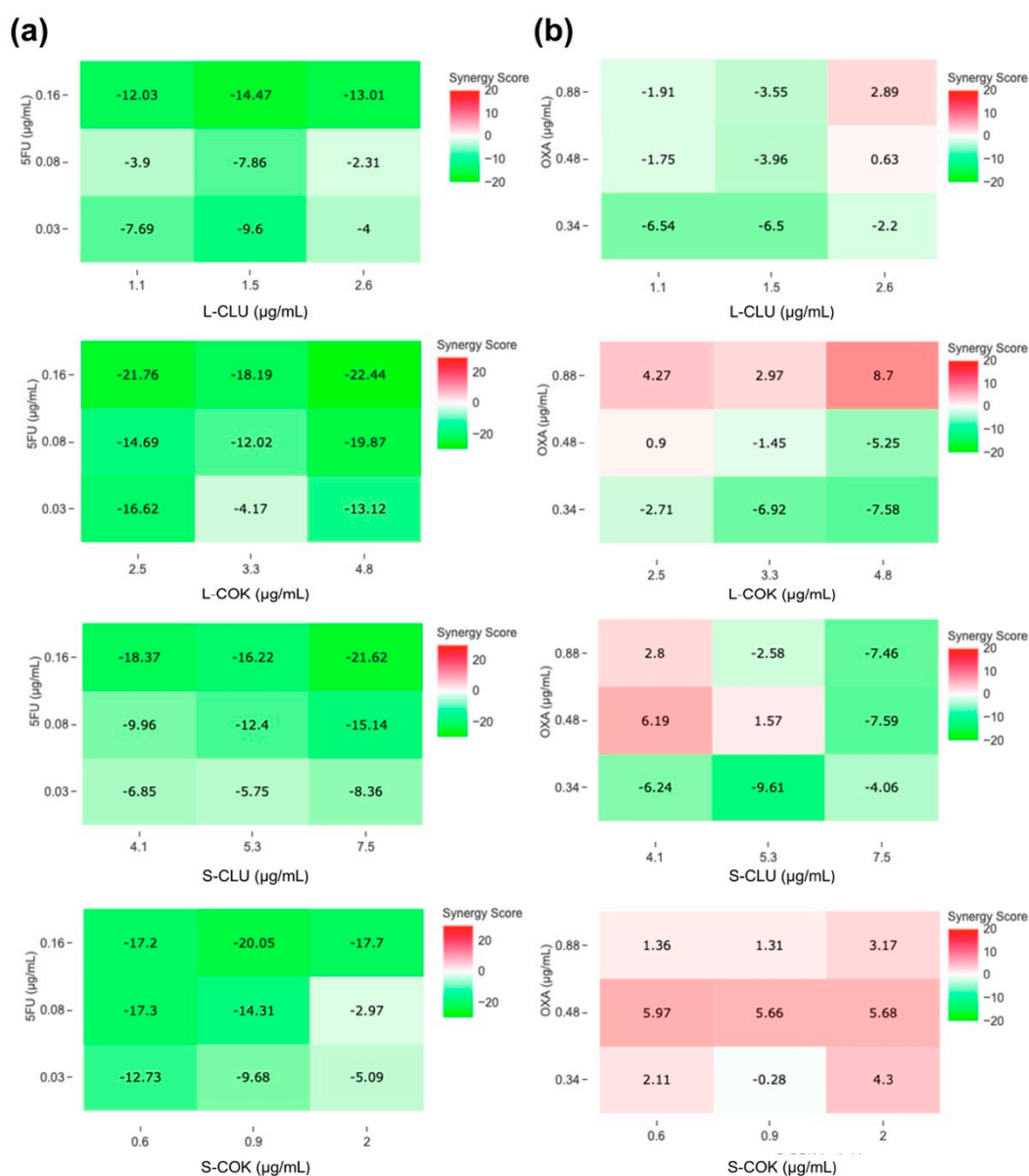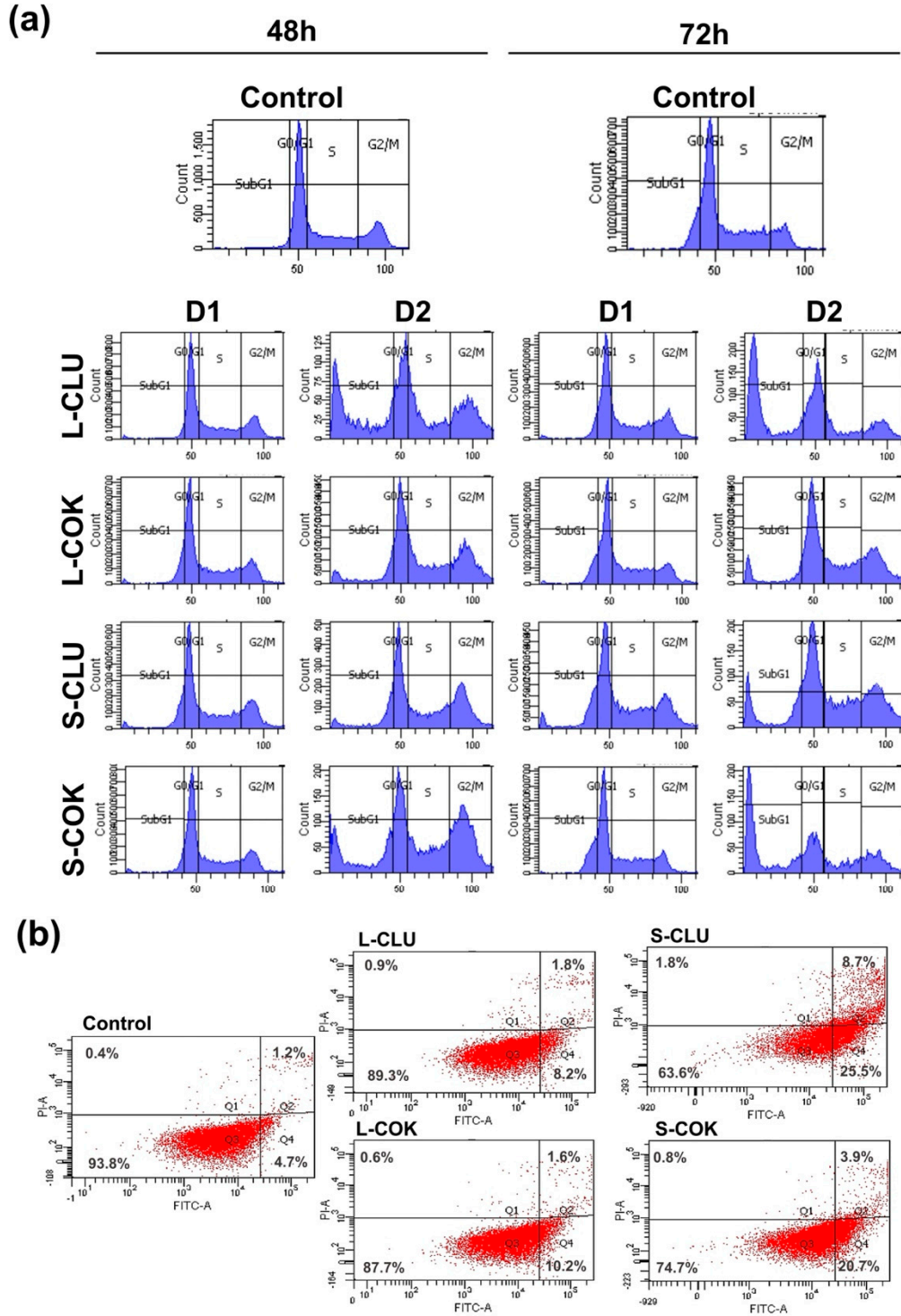
Figure S4. Cell cycle and apoptosis flow cytometry charts. **(a)** T84 cells were treated with two concentrations (D1 and D2) of cEtOH from L-CLU (4 and 11 µg/mL) and S-CLU (11 and 15 µg/mL), L-COK (9 and 15 µg/mL) and S-COK (4 or 11 µg/mL) for 48 and 72 hours for cell cycle study. **(b)** For apoptosis assay, T84 cells were treated with cEtOH from leaves and seeds of CLU (2.5 and 11 µg/mL, respectively) and COK (4.8 and 2 µg/mL, respectively) for 48 hours. Results are represented as dot plots with cell population distribution according to Annexin V-FITC and PI detection: necrotic (Q1), late apoptotic or dead (Q2), live (Q3) or early apoptotic (Q4) cells.
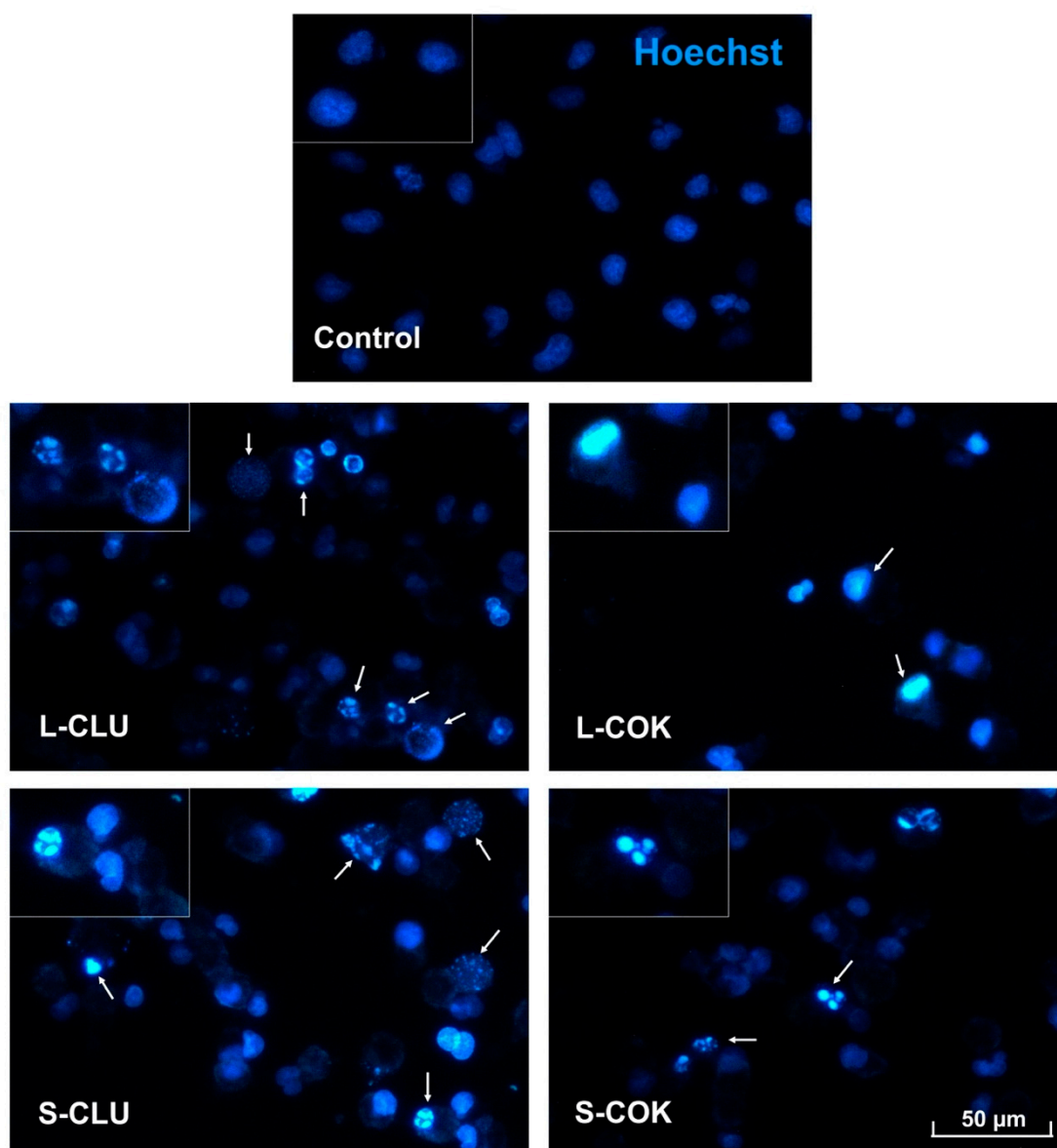
Figure S5. Fluorescence images of T84 cells nuclei after treatment with cEtOH extracts from CLU and COK plants. Cells were treated with L- and S-CLU (30 and 40 µg/mL, respectively) and L- and S-COK (30 and 20 µg/mL, respectively) for 24 hours. Nuclei were stained with Hoechst and images were obtained by fluorescence microscopy (amplified from 20× magnification). Arrows indicate apoptotic nuclear morphology including chromatin condensation, nuclear shrinkage and apoptotic bodies.
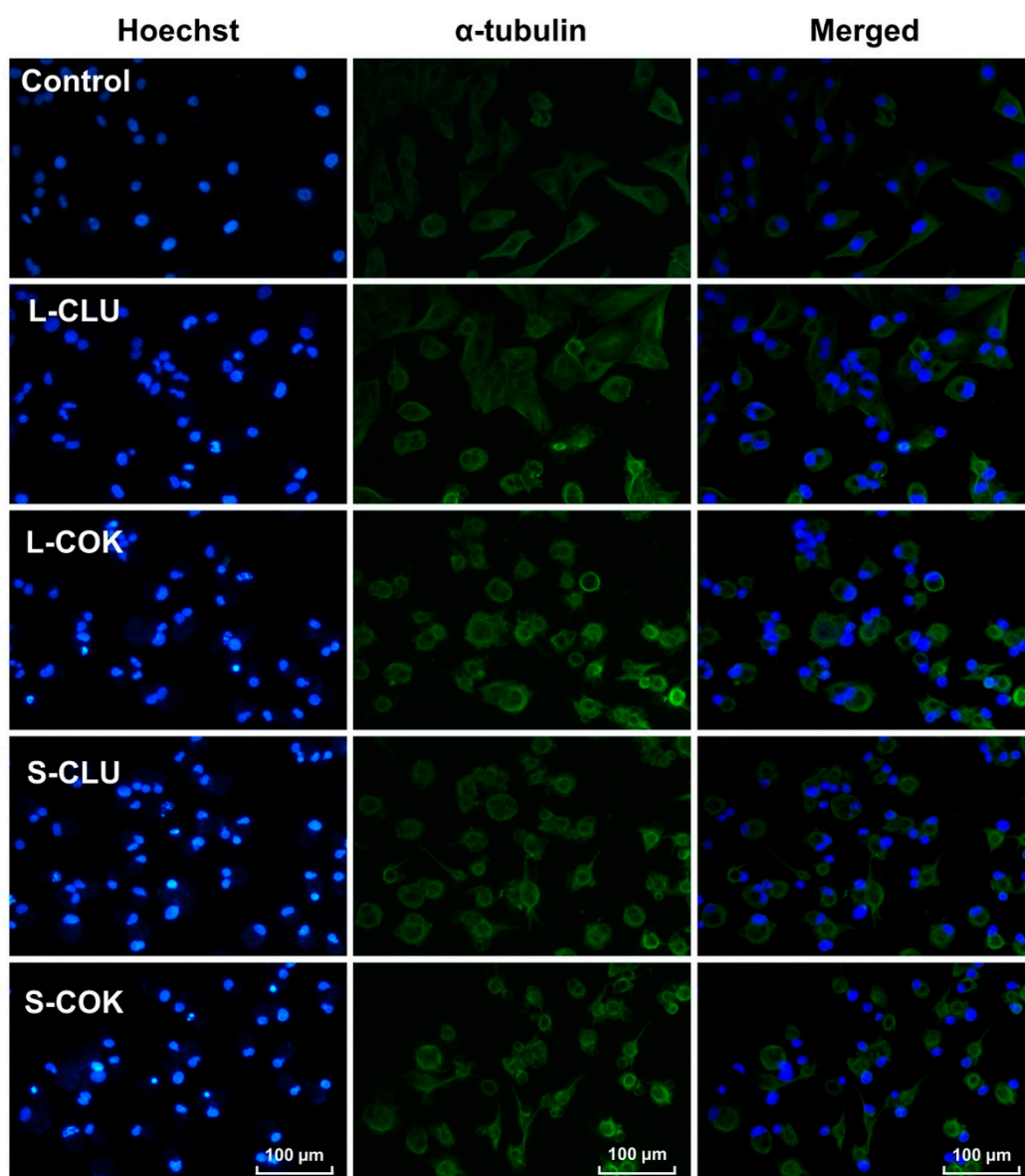
Figure S6. Immunofluorescence stain of α-tubulin in T84 cells treated with cEtOH from CLU and COK plants. T84 CRC cells were treated with cEtOH from L- and S-CLU (12 and 33 µg/mL, respectively) and L- and S-COK (27 and 12 µg/mL, respectively). α-tubulin was stained green and Hoechst 33342 in blue for nuclear localization in fluorescence microscopy images (20× magnification).
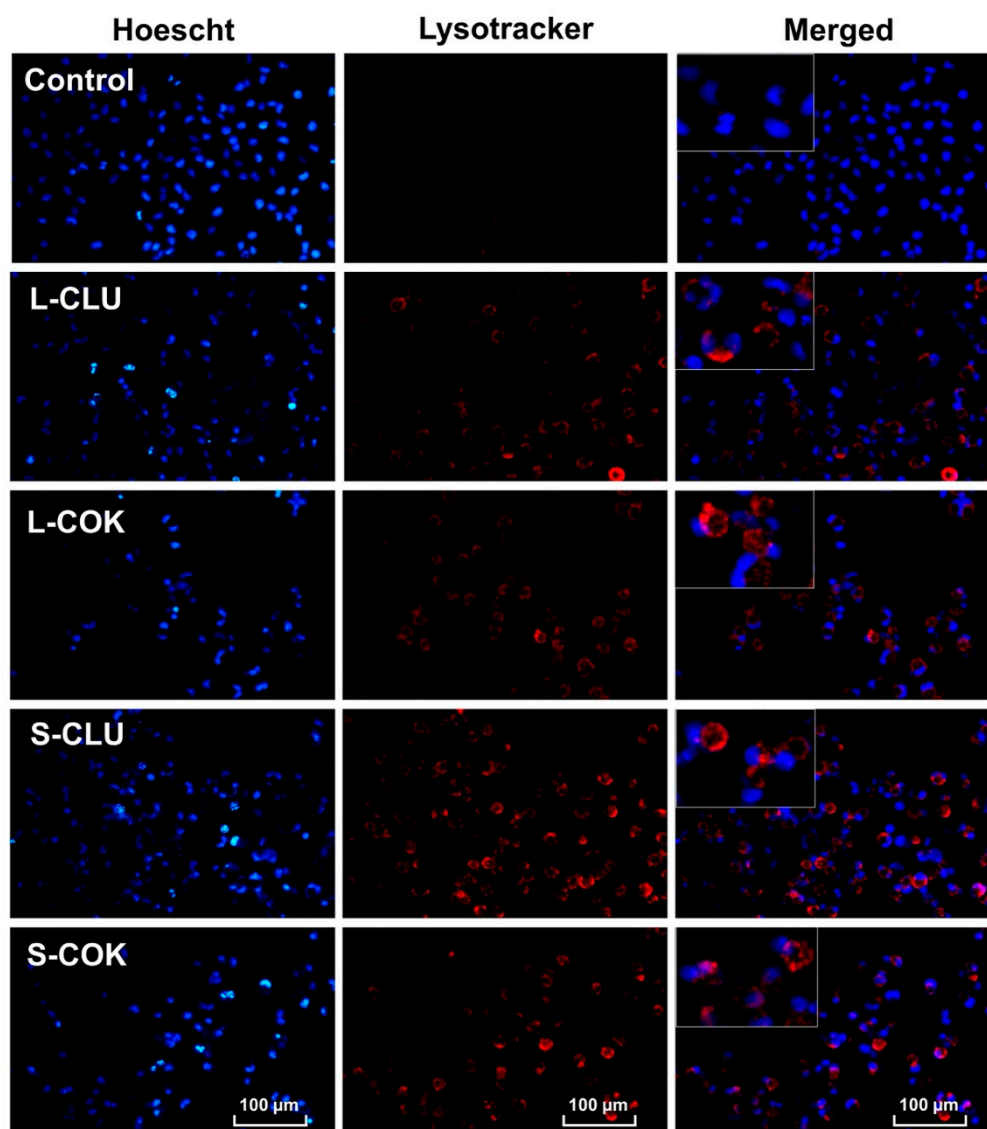
Figure S7. Fluorescence detection of autophagic vesicles in T84 cells treated with cEtOH from CLU and COK plants. T84 cells were treated with cEtOH from L- and S-CLU (30 and 40 μg/mL, respectively) and L- and S-COK (30 and 20 μg/mL, respectively) for 24h. The autophagic vesicles were labeled with Lysotracker in red color and the nuclei were labeled with Hoechst 33342 in blue color (20× magnification).
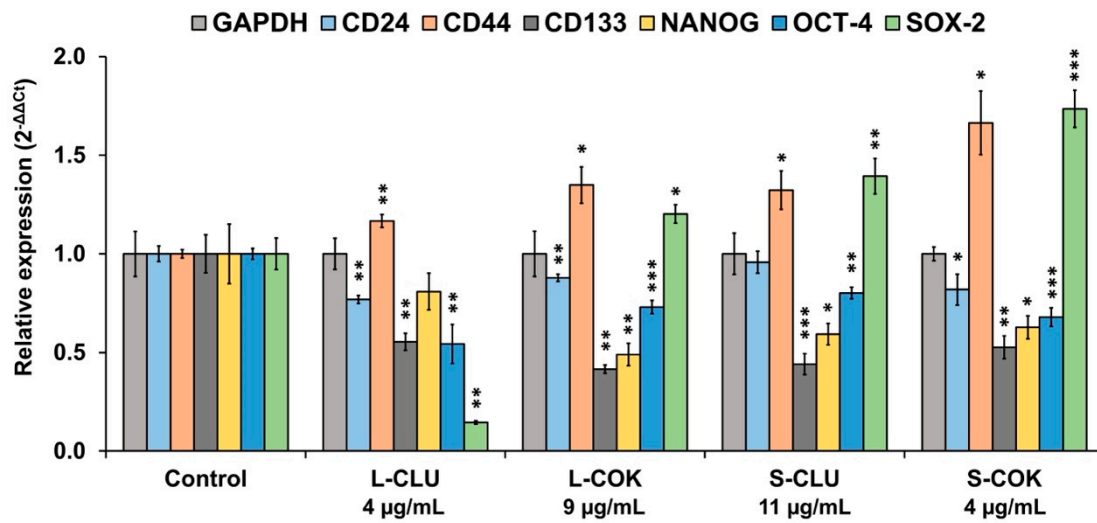
Figure S8. CSC-related genes relative expression in T-84 treated with cEtOH extracts from CLU and COK plants. T-84 cells were exposed to IC_{50} of cEtOH from leaves (L) and seeds (S) of CLU and COK for 72 h. Statistical significance compared to control cells: *p* value < 0.05 (*); < 0.01 (**); < 0.001 (***).
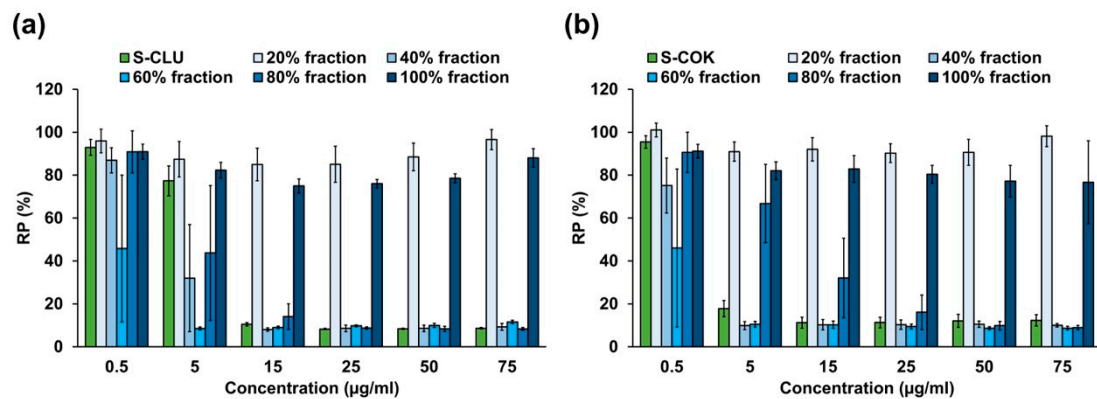
Figure S9. Percentage of relative proliferation of T-84 cells treated with fractions from CLU and COK seeds extracts. T84 CRC cells were treated with increasing concentrations of fractions obtained from **(a)** S-CLU and **(b)** S-COK extracts for 72 h. Data were presented as the mean ± SD of 3 replicates from two independent fractionations.
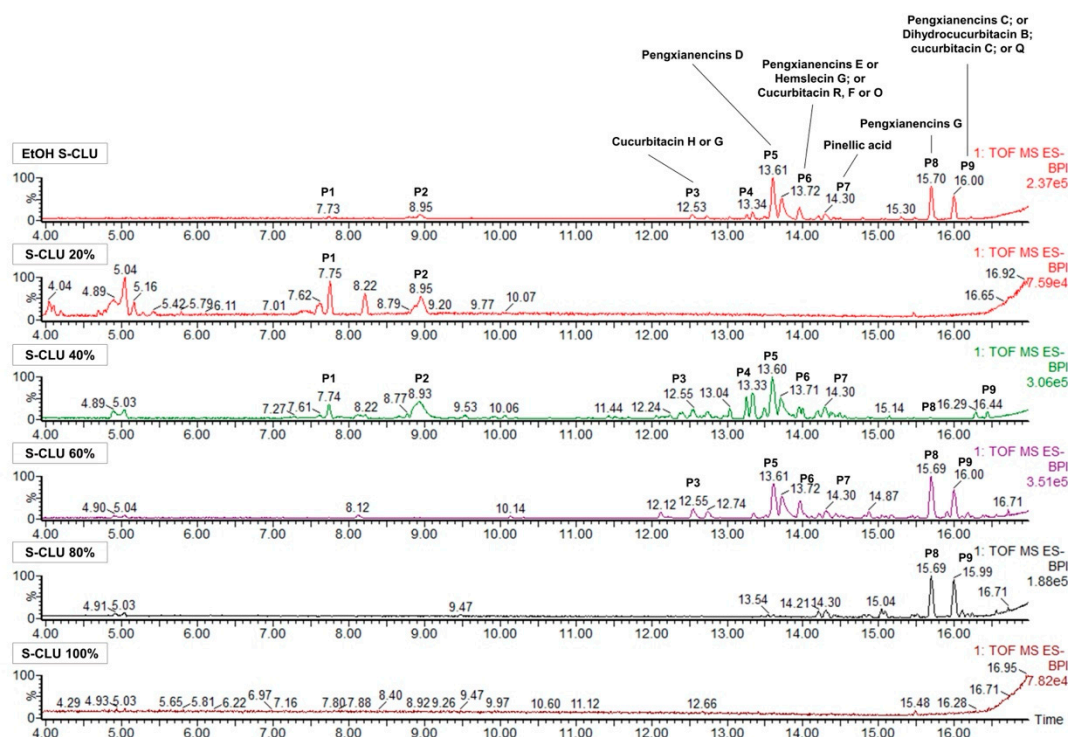
Figure S10. UPLC-MS chromatographic profiles of chromatographic fractions obtained from cEtOH of S-CLU. The highest peaks were highlighted and tentatively identified in mass spectrometry databases such as Dictionary of Natural Products and the Chemspider database.
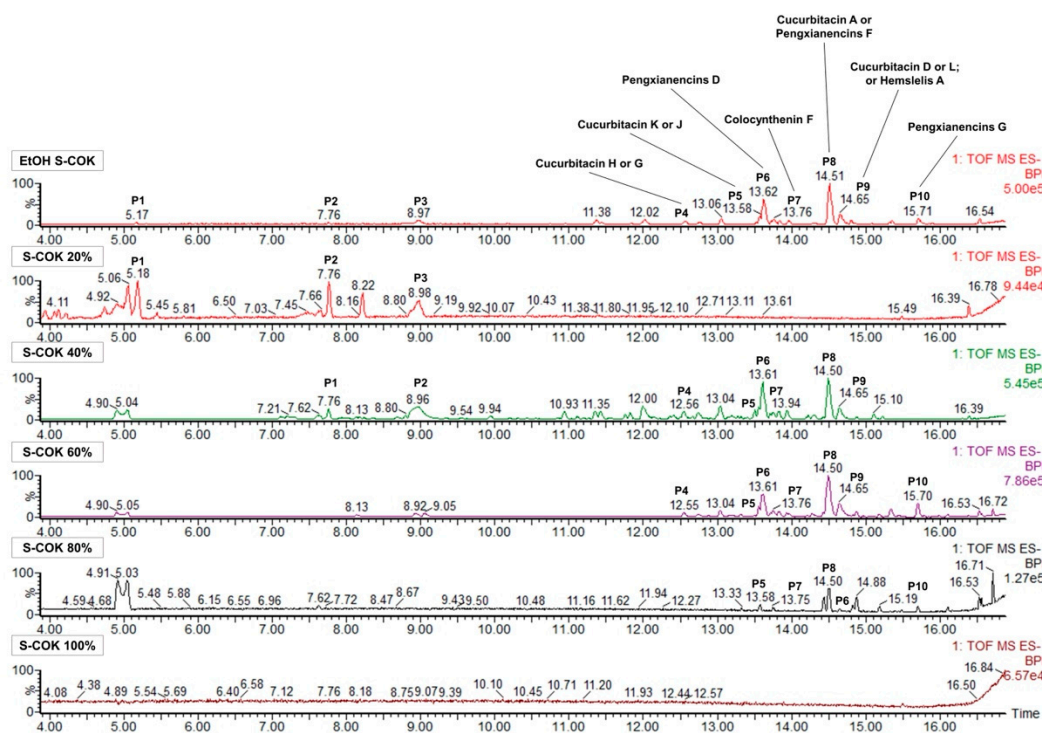
Figure S11. UPLC-MS chromatographic profiles of chromatographic fractions obtained from cEtOH of S-COK. The highest peaks were highlighted and tentatively identified in mass spectrometry databases such as Dictionary of Natural Products and the Chemspider database.

Table S1. Primary and secondary antibodies used in the western blot experiments

Type	Target	Origin	Dilution	Commercial reference
Primary antibodies	Caspase 8	Mouse	1:1000	MA1-41280, Invitrogen
	Caspase 9	Mouse	1:500	9508, Cell Signaling Technology
	PARP1	Rabbit	1:1000	ab32138, abcam
	VEGFA	Rabbit	1:1000	ab46154, abcam
	LC3B	Rabbit	1:1000	NB100-2220, Novus Biologicals
	γ-H2AX (Ser139)	Mouse	1:1000	MA12022, Invitrogen
HRP-conjugated IgG secondary antibodies	Mouse primary antibodies	Horse	1:2000	7076, Cell Signaling Technology
	Rabbit primary antibodies	Goat	1:2000	7074, Cell Signaling Technology
Peroxidase antibody	β-actin	Mouse	1:25000	A3854, Sigma Aldrich

* γ-H2AX (phosphorylated H2A histone family member); HRP (horseradish peroxidase); LC3B (microtubule-associated proteins 1A/1B light chain 3B); PARP1 (poly [ADP-ribose] Polymerase 1); VEGFA (vascular endothelial growth factor A).

Table S2. Primers used in the qPCR experiments.

Gene	Primer	DNA sequence	Tm (°C)
GAPDH	Forward	TCGGAGTCAACGGATTTG	62.4
	Reverse	CAACAATATCCACTTTACCAGAG	59.1
CD24	Forward	CAGTAGTCTTGATGACCAAAG	56.4
	Reverse	ACAGCATTCTGGAATAAAGC	58.4
CD44	Forward	TTATCAGGAGACCAAGACAC	56.6
	Reverse	ATCAGCCATTCTGGAATTTG	61.5
CD133	Forward	AAGCATTGGCATCTTCTATG	59.3
	Reverse	TTTGCTCTGGAGTTTCATTC	59.4
OCT-4	Forward	GATCACCCTGGATATACAC	58.1
	Reverse	GCTTTGCATATCTCCTGAAG	59.1
SOX2	Forward	ATAATAACAATCATCGGCGG	61.1
	Reverse	AAAAAGAGAGAGGCAAACTG	57.8
NANOG	Forward	CCAGAACCAGAGAATGAAATC	60.1
	Reverse	TGGTGGTAGGAAGAGTAAAG	55.9

Table S3. Experimental parameters of the two different UPLC-MS analysis.

Samples	cEtOH L-CLU, L-COK, S-CLU and S-COK	cEtOH S-CLU and S-COK, and their respective fractions
UPLC equipment	ACQUITY UPLC I CLASS (Waters)	ACQUITY UPLC H CLASS (Waters)
Mass Spectrometer	LCT Premier	QTOF MS SYNAP-G2 (Waters)
Column	ACQUITY UPLC HSS T3 (100Å, 1.8 μm, 2.1 mm X 100 mm) (Waters)	ACQUITY UPLC BEH Shield RP18 (130Å, 1.7 μm, 2.1 mm X 100 mm) (Waters)
Injection volumn (μL)	10	10
Flow rate (mL/min)	0.4	0.7
Solvent A	dH$_2$O with 0.5% acetic acid	dH$_2$O with 1% acetic acid
Solvent B	Acetonitrile	Acetonitrile
Gradient	Initial - 95% A; 5% B 15.00 min - 5% A; 95% B 15.10 min - 95% A; 5% B	Initial - 99% A; 1% B 2.33 min - 99% A; 1% B 4.37 min - 93% A; 7% B 8.11 min - 86% A; 14% B 12.19 min - 76% A; 24% B 15.99 min - 60% A; 40% B 18.31 min - 2% A; 98% B 21.83 min - 2% A; 98% B 22.39 min - 99% A; 1% B 25.00 min - 99% A; 1% B
Total run time (min)	18	28
Resolution (FWHM)	13,500	20,000
ESI Mode	Negative	Negative
Mass range (Da)	100 to 1,200	50 to 1,500

Table S4. UPLC-MS peak tentative identification in cEtOH obtained from leaves and seeds of CLU and COK.

Peak	Sample	RT (min)	[M-H]-	Error (PPM)	% Conf	Suggested MF	Suggested Compounds Common Name	MS fragments	Classification
P1	L-CLU	2.952	355.0669	1.1	99.88	C15H16O10	Caffeic acid 3-O-glucuronide	173.0076; 151.0622; 120.0387	Phenolic acid / Phenolic Compound
	L-CLU	2.952	711.1472	4.9	97.53	C30H32O20	Quercetin 3-O-(6"-malonyl-glucoside) 7-O-glucoside	306.0469; 273.0515; 173.0071	Flavonoid / Phenolic Compound
P2	L-CLU	3.583	353.0536	7.6	99.85	C15H14O10	2-caffeoylisocitric acid	189.0028; 159.0601; 141.0243	Phenolic acid; Phenolic Compound
	L-COK	3.582	353.0528	5.4	99.96			173.0071; 158.9921; 119.0354	
P3	L-CLU	4.175	337.0545	-4.5	99.96	C15H14O9	7-Hydroxycoumarin glucuronide	173.0074; 119.0357; 112.9865	Hydroxycoumarin / Phenolic Compound
	L-COK	4.174	337.0539	-6.2	93.05			306.0468; 273.0532; 173.0078	
P4	L-CLU	4.525	367.0635	-8.2	99.33	C16H16O10	Scopoletin b-D-glucuronide	173.0085; 119.0357; 112.9864	Hydroxycoumarin / Phenolic Compound
	L-COK	4.49	367.0626	-10.6	97.55			306.0464; 219.0862; 173.0067	
	L-CLU	4.525	463.0882	1.1	99.5	C21H20O12	Isoquercetin	173.0085; 119.0357; 112.9864	Flavonoid /Phenolic Compound
P5	L-CLU	4.773	549.0915	6.4	99.12	C24H22O15	Quercetin 3-O-malonylglucoside	273.0546; 173.0088; 119.0357	Flavonoid / Phenolic Compound
	L-COK	4.771	549.0894	2.5	98.8			306.0468; 219.0864; 173.0065	
P6	L-CLU	5.015	447.0925	-0.4	99.69	C21H20O11	Quercitrin	306.0462; 173.0081; 119.0358	Flavonoid / Phenolic Compound
	L-COK	5.014	447.0933	1.3	98.27			306.0451; 219.0865; 173.0071	

Peak	Sample	RT (min)	[M-H]-	Error (PPM)	% Conf	Suggested MF	Suggested Compounds Common Name	MS fragments	Classification
P7	L-CLU	5.399	563.1028	-1.6	99.61	C25H24O15	Isorhamnetin 3-(6''-malonylglucoside)	306.0459; 219.0880; 173.0081	Flavonoid / Phenolic Compound
	L-COK	5.363	563.1022	-2.7	99.24			306.0462; 173.0836; 119.0350	
P8	L-CLU	7.253	327.2161	-3.1	99.66	C18H32O5	12,13,16-Trihydroxy-9,14-octadecadienoic acid derivates/	-	Oxylipins
	L-COK	7.218	327.2176	1.5	99.81		12,15,16-Trihydroxy-9,13-octadecadienoic acid derivates	-	
	S-CLU	7.16	327.2169	-0.6	99.49			-	
P9	L-CLU	7.569	779.3893	5	92.61	C40H60O15	-	-	Steroids
P10	L-CLU	8.86	589.3236	2	99.05	C29H50O12	-	-	Carbohydrate
P11	L-CLU	9.525	617.3359	5.3	99.12	C34H50O10	Pengxianencins G	N.A.	Cucurbitane-type triterpenoid
	L-COK	9.525	617.3344	2.9	99.25			N.A.	
	S-CLU	9.262	617.3356	4.9	76.73			N.A.	
	S-COK	9.257	617.3352	4.2	98.57			N.A.	
P12	L-CLU	10.19	615.3182	2.1	99.55	C34H48O10	-	-	Terpenoid
	L-COK	10.19	615.3182	2.1	99.4			-	
	S-COK	9.92	615.319	3.4	99.12			-	
P13	L-CLU	11.098	899.5381	1.4	86.39	C47H80O16	Dammar-24-ene-3,20,21-triol; (3β,20S)-form; 3-O-[α-L-Rhamnopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-glucopyranoside]	N.A.	Dammarane-type triterpenoid
	L-COK	11.064	899.5405	4.1	85.86			N.A.	
P14	L-CLU	11.341	587.4313	0.2	99.49	C36H60O6	26,27-Dimethyl-26-methylenecholest-5-en-3-ol; (3β,25ξ)-form, 3-O-β-D-Glucopyranoside	N.A.	Cholestane steroid
	L-COK	11.34	587.429	-3.7	90.4			N.A.	
	S-CLU	11.26	587.4299	-2.2	91.25			N.A.	
	S-COK	11.11	587.4312	0	97.02			N.A.	
P15	L-CLU	11.656	1087.6431	-1.2	99.27	C29H50O11	-	-	-

Peak	Sample	RT (min)	[M-H]-	Error (PPM)	% Conf	Suggested MF	Common Name	MS fragments	Classification
	L-COK	11.656	573.3294	3.3	99.83			-	
P16	L-CLU	12.461	431.2215	-15.3	83.11	C21H36O9	-	-	Terpenoid
	L-COK	12.738	431.2196	-19.7	70.35			-	
P17	L-CLU	13.51	411.2747	0.0	99.7	C23H40O6	-	-	Terpenoid
	L-COK	13.51	411.2748	0.2	99.92			-	
P18	L-CLU	14.209	865.5347	3.9	88.46	C47H78O14	-	-	-
P19	L-CLU	14.384	417.3212	-1	99.9	C23H46O6	-	-	Aliphatic natural product
	L-COK	14.417	417.3209	-1.7	99.73			-	
	S-COK	13.81	417.3196	-4.8	97.93			-	
P20	L-CLU	14.593	555.442	1.3	99.65	C36H60O4	-	-	-
	L-COK	14.592	555.442	1.3	94.63			-	
P21	L-COK	6.17	765.2615	1.2	98	C36H46O18	-	-	-
P22	L-COK	7.393	655.4454	5	98.06	C36H64O10	-	-	Dammarane-type triterpenoid
P23	L-COK	8.369	573.3074	1.7	99.68	C32H46O9	Cucurbitacin A / Pengxianencins F	(559.2914; 293.1777; 159.0620) / N.A.	Cucurbitane-type triterpenoid
	S-COK	8.11	573.3056	-1.4	99.97			(560.2944; 559.2905; 515.2968) / N.A.	
P24	L-COK	10.083	619.4231	3.4	98.98	C36H60O8	Balsaminoside B	(389.2831; 353.2831; 119.0356)	Cucurbitane-type triterpenoid
P25	S-CLU	4.6	733.3333	6.8	85.85	C34H54O17	-	-	-
	S-COK	4.6	733.3306	3.1	70.26			-	
P26	S-CLU	5.86	979.4788	3.9	73.81	C46H76O22	-	-	-
P27	S-CLU	6.04	981.4936	3.1	89.66	C46H78O22	-	-	-
P28	S-CLU	6.71	593.3326	0	96.19	C32H50O10	-	-	-
	S-COK	6.81	593.332	-1	99.17			-	
P29	S-CLU	6.92	493.2078	0.8	99.76	C25H34O10	-	-	-

Peak	Sample	RT (min)	[M-H]-	Error (PPM)	% Conf	Suggested MF	Suggested Compounds Common Name	MS fragments	Classification
P30	S-CLU	7.509	575.3215	-0.9	97.65	C32H48O9	Pengxianencins D/ 23,24-dihydrocucurbitacin A / 7-hydroxycucurbitacin F-25-O-acetate	N.A. / (400.2678) / (400.2678)	Cucurbitane-type triterpenoid
	S-COK	7.507	575.3212	-1.4	99.74			N.A. / (414.2766; 400.2586; 196.0661) / (414.2766; 400.2586; 196.0661)	
P31	S-CLU	8.28	493.3166	0.2	99.71	C28H46O7	-	-	-
P32	S-CLU	9.51	619.3475	8.4	96.97	C41H48O5	-	-	-
P33	S-CLU	11.96	295.2278	1.7	N.A.	C18H32O3	18-Hydroxylinoleic acid	-	Fatty acid
	S-COK	11.96	295.2254	-6.4	N.A.			-	
P34	S-CLU	12.41	297.243	0	N.A.	C18H34O3	Ricinoleic acid	-	Fatty acid
	S-COK	12.41	297.2425	-1.7	N.A.			-	
P35	S-CLU	14.24	413.2883	-4.8	87.75	C23H42O6	-	-	-
	S-COK	14.23	413.2906	0.7	99.96			-	
P36	S-CLU	15.21	609.5109	2.5	98.43	C37H70O6	-	-	-
	S-COK	15.21	609.51	1	99.1			-	
P37	S-COK	5.83	737.3768	2.7	53.78	C38H58O14	-	-	Steroid
P38	S-COK	6.11	607.3118	0	98.16	C32H48O11	-	-	Steroid
P39	S-COK	7.06	545.2748	-0.6	99.3	C30H42O9	16,20,25-Trihydroxy-11,22-dioxo-2,3-secocucurbita-1(10),5,23-triene-2,3-dioic acid	N.A.	Cucurbitane-type triterpenoid
P40	S-COK	7.41	591.3184	2.5	97.5	C32H48O10	Colocynthenin F	220.0325	Cucurbitane-type triterpenoid
P41	S-COK	9.155	619.3472	-1.6	96.81	C34H52O10	Pengxianencins C	N.A.	Cucurbitane-type triterpenoid

(*) Not available data (N.A.)

Table S5. UPLC-MS peak tentative identification in fractions obtained from cEtOH of S-CLU.

Peak	Sample	RT (min)	[M-H]-	Error (PPM)	% Conf	Suggested MF	Suggested Compounds		
							Common Name	**MS Fragments**	**Classification**
1	EtOH S-CLU	7.73	776.3096	-	-	-	-	-	-
	S-CLU 20%	7.759	776.3135	-	-			-	
	S-CLU 40%	7.737	776.3137	-	-			-	
2	EtOH S-CLU	8.965	673.3038	-4.9	99.69	C32H50O15	-	-	-
	S-CLU 20%	8.950	673.3049	-3.3	85.53			-	
	S-CLU 40%	8.927	673.3045	-3.9	99.99			-	
3	EtOH S-CLU	12.544	533.3116	0.4	84.81	C30H46O8	Cucurbitacin H / G	(428.2518; 427.2499; 341.2148)	Cucurbitane-type triterpenoid
	S-CLU 40%	12.551	533.313	3	99.98			(445.2607; 427.2494; 341.2145)	
	S-CLU 60%	12.551	533.3122	1.5	99.75			(515.3002; 445.2598; 427.2492)	
4	EtOH S-CLU	13.345	493.2069	-1	98.45	C25H34O10	-	-	Terpenoid
	S-CLU 40%	13.337	493.2083	1.8	100			-	
5	EtOH S-CLU	13.607	575.3214	-1	99.79	C32H48O9	Pengxianencins D / 23,24-dihydrocucurbitacin A / 7-hydroxycucurbitacin F-25-O-acetate	N.A. / (515.2995; 497.2920; 327.2170) / (515.2995; 497.2920; 327.2170)	Cucurbitane-type triterpenoid
	S-CLU 40%	13.599	575.3225	0.9	100			N.A. / (515.3018; 497.2920; 328.2229) / (515.3018; 497.2920; 328.2229)	
	S-CLU 60%	13.614	575.3198	-3.8	97.92			N.A. / (515.2999; 497.2805; 327.2184) / (515.2999; 497.2805; 327.2184)	
6	EtOH S-CLU	13.719	577.3374	-0.5	99.92	C32H50O9	Pengxianencins E / Hemslecin G	N.A.	Cucurbitane-type triterpenoid
	S-CLU 40%	13.711	577.3371	-1	99.96			N.A.	
	S-CLU 60%	13.726	577.3345	-5.5	99.99			N.A.	
	EtOH S-CLU	13.719	517.3165	0	99.34	C30H46O7		(499.3076; 385.2386; 327.2186)	

No	Sample	RT	m/z	Error	Match	Formula	Compound	Fragments	Class
	S-CLU 40%	13.711	517.317	1	90.74		Cucurbitacin R / Cucurbitacin F / Cucurbitacin O	(499.3063; 385.2375; 328.2213)	Cucurbitane-type triterpenoid
	S-CLU 60%	13.726	517.3149	-3.1	99.99			(499.3033; 385.2363; 327.2168)	
7	EtOH S-CLU	14.295	329.2348	6.1	N.A.	C18H34O5	Pinellic acid	(211.1336; 171.1048; 127.1117)	Fatty acid
	S-CLU 40%	14.295	329.2342	4.3	N.A.			(229.1462; 211.1358; 171.1039)	
	S-CLU 60%	14.31	329.2346	5.5	N.A.			(229.1459; 211.1359; 171.1048)	
	S-CLU 80%	14.302	329.233	0.6	99.59			(293.2141; 211.1337; 171.1041)	
8	EtOH S-CLU	15.695	617.3322	-0.6	99.99	C34H50O10	Pengxianencins G	N.A.	Cucurbitane-type triterpenoid
	S-CLU 40%	15.68	617.3322	-0.6	99.86			N.A.	
	S-CLU 60%	15.695	617.332	-1	99.95			N.A.	
	S-CLU 80%	15.694	617.33	-4.2	99.95			N.A.	
9	EtOH S-CLU	16.002	619.3452	-4.8	94.01	C34H52O10	Pengxianencins C	N.A.	Cucurbitane-type triterpenoid
	S-CLU 60%	16.002	619.3483	0.2	99.99			N.A.	
	S-CLU 80%	16.001	619.3455	-4.4	99.79			N.A.	
	EtOH S-CLU	16.002	559.3259	-2.1	99.95	C32H48O8	Dihydrocucurbitacin B / Cucurbitacin C / Cucurbitacin Q	(481.2954; 412.2264; 341.2119)	Cucurbitane-type triterpenoid
	S-CLU 40%	16.443	559.3265	-1.1	99.56			(559.3331; 481.2941)	
	S-CLU 60%	16.002	559.3284	2.3	99.92			(481.2971; 412.2282; 341.2150)	
	S-CLU 80%	16.001	559.3251	-3.6	96.33			(481.2954; 412.2262; 341.2122)	

(*) Not available data (N.A.)

Table S6. UPLC-MS peak tentative identification in fractions obtained from cEtOH of S-COK.

Peak	Sample	RT (min)	[M-H]-	Error (PPM)	% Conf	Suggested MF	Suggested Compounds Common Name	MS fragments	Classification
1	EtOH S-COK	5.162	475.1822	1.3	99.41	C21H32O12	-	-	-
	S-COK 20%	5.185	475.181	-1.3	100			-	-
2	EtOH S-COK	7.759	776.314	-	-	-	-	-	-
	S-COK 20%	7.775	776.3128	-	-			-	-
	S-COK 40%	7.759	776.3124	-	-			-	-
3	EtOH S-COK	8.972	673.306	-1.6	100	C32H50O15	-	-	-
	S-COK 20%	8.973	673.3066	-0.7	89.96			-	-
	S-COK 40%	8.973	673.3087	2.4	99.08			-	-
4	EtOH S-COK	12.56	533.3122	1.5	99.97	C30H46O8	Cucurbitacin H / G	(445.2613; 427.2491; 341.2132)	Cucurbitane-type triterpenoid
	S-COK 40%	12.55	533.3112	-0.2	99.78			(445.2601; 427.2484; 341.2130)	
	S-COK 60%	12.54	533.3101	-2.4	99.98			(445.2578; 427.2479; 341.2120)	
5	EtOH S-COK	13.57	531.2959	0.2	99.24	C30H44O8	Cucurbitacin K / Cucurbitacin J	(443.2434; 425.2333; 383.2216)	Cucurbitane-type triterpenoid
	S-COK 40%	13.55	531.295	-1.5	96.7			(443.2439; 425.2325; 328.2217)	
	S-COK 60%	13.56	531.2961	0.6	100			(443.2434; 425.2325; 327.2181)	
	S-COK 80%	13.58	531.295	-1.5	97.87			(426.2373; 425.2333; 339.1988)	
6	EtOH S-COK	13.63	575.3215	-0.9	99.09	C32H48O9	Pengxianencins D / 23,24-dihydrocucurbitacin A / 7-hydroxycucurbitacin F-25-O-acetate	N.A. / (515.3011; 425.2340; 327.2191) / (515.3011; 479.2796; 327.2191)	Cucurbitane-type triterpenoid
	S-COK 40%	13.61	575.3214	-1	99.52			N.A. / (515.3015; 479.2809; 327.2184) / (515.3015; 479.2809; 327.2184)	

	Sample	RT	m/z	Error	Purity	Formula	Compound	Fragments	Type
	S-COK 60%	13.62	575.3215	-0.9	99.97			N.A. / (515.3008; 425.2322; 327.2174) / (515.3008; 479.2795; 327.2174)	
	S-COK 80%	14.65	575.3215	-0.9	98.18			N.A. / (426.2336; 425.2316; 339.1933)	
7	EtOH S-COK	13.76	591.3163	-1	91.25	C32H48O10	Colocynthenin F	(513.2854; 443.2435; 425.2327)	Cucurbitane-type triterpenoid
	S-COK 60%	13.76	591.3173	0.7	99.63			(531.2969; 443.2447; 425.2335)	
	S-COK 80%	13.75	591.3159	-1.7	97.6			(513.2862; 443.2451; 425.2329)	
8	EtOH S-COK	14.51	573.3066	0.3	99.97	C32H46O9	Cucurbitacin A / Pengxianencins F	(513.2863; 495.2759; 477.2650) / N.A.	Cucurbitane-type triterpenoid
	S-COK 40%	14.5	573.3052	-1.2	99.89			(513.2848; 495.2741; 477.2631) / N.A.	
	S-COK 60%	14.48	573.3055	-1.6	99.93			(513.2850; 477.2639; 383.2224) / N.A.	
	S-COK 80%	14.5	573.3055	-1.6	20.72			(513.2883; 495.2753; 435.2565) / N.A.	
9	EtOH S-COK	14.65	515.3009	0	99.3	C30H44O7	Cucurbitacin D / Cucurbitacin L	(497.2910; 384.2258; 383.2221) / (455.2805; 383.2221; 339.1972)	Cucurbitane-type triterpenoid
	S-COK 40%	14.65	515.3011	0.4	99.99			(497.2896; 383.2224; 325.2030) / (455.2792; 383.2224; 325.2030)	
	S-COK 60%	14.65	515.2999	-1.9	99.6			(497.2893; 455.2798; 383.2224) / (455.2798; 383.2224; 327.2182)	
10	EtOH S-COK	15.72	617.3306	-3.2	48.44	C34H50O10	Pengxianencins G	N.A.	Cucurbitane-type triterpenoid
	S-COK 60%	15.7	617.3306	-3.2	99.82			N.A.	
	S-COK 80%	15.7	617.3329	0.5	79.17			N.A.	

(*) Not available data (N.A.)