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Supplementary Material

Cyclocodon lancifolius fruit prolongs the lifespan of *Caenorhabditis elegans* via antioxidation and regulation of purine metabolism

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Table S1. Primers used for qRT-PCR assays.

Gene's name	Forward (5′-3′)	Reverse (5′-3′)
daf-16	CCACCACCATCATACCACGAGTTG	GAAGAGCCGATGAAGAAGCGACAG
daf-2	CCACGACGACGAGCACATCAC	ATTGTCAGCGAACCTTCCACCAC
mtl-1	TCATGGCTTGCAAGTGTGACT	TGATGGGTCTTGTCTCCGCA
age-1	GGAGCAAGGATAGGCACCAACG	GCGAGTTCGGAGAGCACAATGAG
skn-1	TTCACCATCGTCCAACACCTCAATC	GATCATCACGCCAACGGAGACC
sod-3	TCATGCCACCTACGTGAACAATCTC	AGAGCCTTGAACCGCAATAGTGATG
hsp-16.2	TGCCATCAATCTCAACGTCTCACAG	CTTCGACGATTGCCTGTTGAATTGG
akt-1	CGCGAAGAAGTTGCTCACAC	GATCACCGCCAATGGCAAAC
xdh-1	TCTCCGCTAACTCCTGTCTTATGC	TGAACTGGATGAAGCCGATTCTTG
guk-1	CAAAGCGGAAGCACGGACTG	GGCGAGAACTGAGGAGGAAGG
F45F2.9	CAGTTCTTCACGAGCACAGTAGG	TTCATTACTCCGCTTTGTTGATTCG
Y71H10B.1	CTCGTCATAAGCCTGTCGTCAAG	AACATCCATACTCCTCGTCCATTTG
R09H10.3	ATGGGCGTGTTGATTGGGTATC	TGTAGTATGGTTCCGTGATGTAGAC
ZK697.8	CAACATTCGCAACGCTACACAAC	ATGATCCACGGTAGGTAGAGTATCC
ampd-1	TTACGGAAGGAGCAAGAACGAATG	CGAGGAATCTGAACGAGCCATC
β-action	ATCCATCGTTCACCGCAAGT	TAAGGACAAAAATGGGGCGG

Table S2. Survival time of *C. elegans*.

Treatment	Median time	Maximum time	Number
Control (Normal)	15.6±0.3 [a]	19.7±0.6 [a]	180
CF-L (Normal)	17.5±0.5 [b]	21.6±1.6 [b]	182
CF-M (Normal)	16.5±0.5 [b]	23.3±0.6 [b]	176
CF-H (Normal)	17.1±0.3 [b]	24.7±0.6 [b]	175
Positive (Normal)	17.5±0.5 [b]	24.7±0.6 [b]	180
Control (H_2O_2)	2.0±0.5 [a]	4.2±0.3 [a]	174
CF-L (H_2O_2)	2.2±0.3 [a]	5.3±0.3 [a]	180
CF-M (H_2O_2)	2.7±0.3 [b]	6.0±0.5 [b]	180
CF-H (H_2O_2)	3.0±0.5 [a]	6.5±0.5 [b]	178
Positive (H_2O_2)	2.8±0.8 [b]	6.7±0.3 [b]	176
Control (Juglone)	2.5±0.5 [a]	10.0±1.0 [a]	181
CF-L (Juglone)	3.5±0.5 [b]	11.0±1.0 [b]	174
CF-M (Juglone)	5.2±0.3 [b]	12.0±1.0 [b]	176
CF-H (Juglone)	6.0±0.5 [b]	13.7±0.6 [b]	174
Positive (Juglone)	5.2±0.3 [b]	13.3±0.6 [b]	177
Control (Heat)	18.2±1.0 [a]	21.0±1.0 [a]	180
CF-L (Heat)	20.8±1.0 [b]	25.0±1.0 [b]	177
CF-M (Heat)	21.0±1.0 [b]	26.7±1.5 [b]	175
CF-H (Heat)	22.8±0.8 [b]	28.0±1.0 [b]	181
Positive (Heat)	22.8±0.8 [b]	29.7±1.5 [b]	180
Control (Ultraviolet)	5.8±0.3 [a]	9.7±0.6 [a]	180
CF-L(Ultraviolet)	7.7±0.6 [b]	12.0±1.0 [b]	176
CF-M (Ultraviolet)	7.5±1.3 [b]	12.3±0.6 [b]	174
CF-H (Ultraviolet)	9.2±0.8 [b]	14.0±1.0 [b]	177
Positive (Ultraviolet)	9.5±0.5 [b]	14.7±0.6 [b]	175

Note: All data are presented as mean ± SD (n = 3). Significant differences ($p < 0.05$) between the mean values denoted by different letters in the same column. Comparisons between treatment and control groups under the same treatment condition were made (One-way ANOVA).

Table S3. Key metabolites in *C. elegans* relevant to CF treatment.

KEGG ID	Metabolite name	RT（min)	Formula	*m/z*	Class
C00357	N-Acetyl-D-Glucosamine 6-Phosphate	1.18	$C_8H_{16}NO_9P$	300.05	Carbohydrate
C00362	dGMP	1.38	$C_{10}H_{14}N_5O_7P$	346.06	Carbohydrate
C00777	All-trans-retinoic acid	1.88	$C_20H_{28}O_2$	217.00	Terpenoid
C00366	Uric acid	2.00	$C_5H4N_4O_3$	169.04	Alkaloid
C03672	Hydroxyphenyllactic acid	8.81	$C_9H_{10}O_4$	181.05	Shikimate and Phenylpropanoid
C08493	1H-Indole-3-carboxaldehyde	8.85	C_9H_7NO	146.06	Alkaloid
C03264	D-Leucic acid	10.23	$C_6H_{12}O_3$	131.07	Amino acid
C01727	Lumichrome	11.90	$C_{12}H_{10}N_4O_2$	243.09	Alkaloid
C12287	beta-Ionone	15.06	$C_{13}H_{20}O$	193.16	Shikimate and Phenylpropanoid
C05608	(E)-3-(4-Hydroxyphenyl)-2-propenal	16.65	$C_9H_8O_2$	149.06	Shikimate and Phenylpropanoid
C02752	4-Hydroxy-6-methyl-2-pyrone	29.65	$C_6H_6O_3$	127.04	Poiyketide
C02814	Benzene-1,2,4-triol	29.77	$C_6H_6O_3$	127.04	Terpenoid
C02154	Glyceraldehyde	29.82	$C_3H_6O_3$	139.02	Carbohydrate

Table S4 Key enzymes and corresponding genes in the purine metabolic pathway enriched by FELLA

EC number	p score	EC name	Gene ID (symbol)
1.17.1.4	0.000001	xanthine dehydrogenase	178381 (*xdh-1)*
2.7.4.8	0.000001	guanylate kinase	171963 (*guk-1*)
3.1.3.89	0.000001	5'-deoxynucleotidase	185809 (*F45F2.9*)
3.1.3.5	2.21259E-05	5'-nucleotidase	180573 (*Y71H10B.1*)
1.17.3.2	0.005230038	xanthine oxidase	178381 (*xdh-1*)
3.5.2.17	0.021952491	hydroxyisourate hydrolase	187757 (*R09H10.3*); 191414 (*ZK697.8*)
3.5.4.6	0.02810366	AMP deaminase	173891 (*ampd-1*)

Fig. S1 *Cyclocodon lancifolius* (Roxb.) Kurz fruit

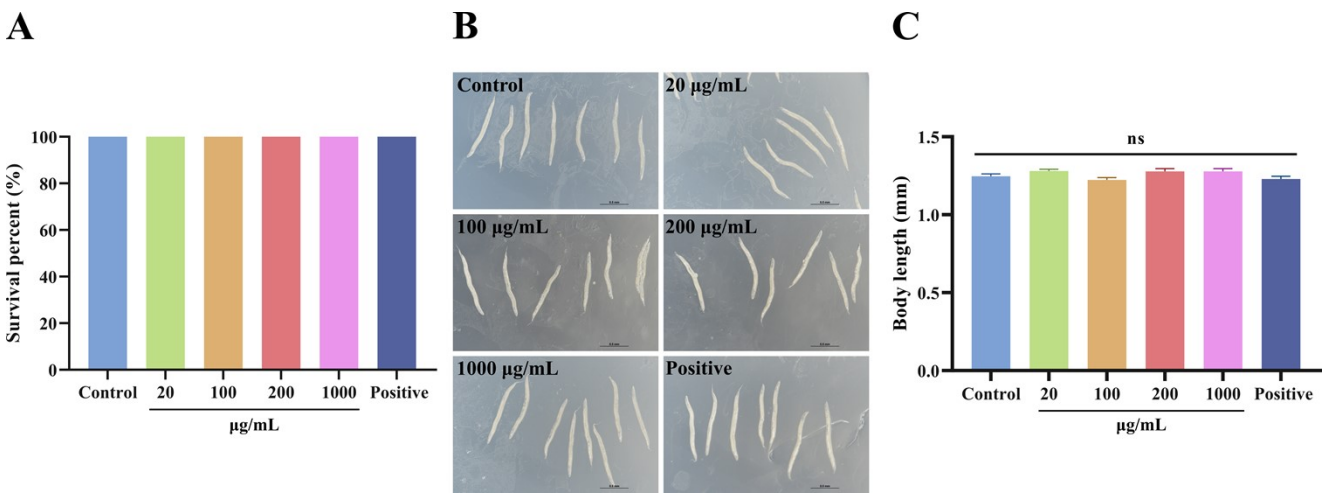

Fig. S2 Toxic effects of CF on *C. elegans*. (A) Acute toxicity of CF at different concentrations on *C. elegans* (30 worms per group, n = 3). (B) Images of worms cultured with or without different concentrations of CF for 5 days. (C) Statistical analysis of body length of worms in each group of B ($n \geq 30$). Worms were cultured on NGM plates containing different concentrations of CF (20, 100, 200 and 1000 μg/mL). No CF treatment was used as a control and treated with resveratrol (100 μM) was a positive control. Results are presented as means ± SEM, ns = no significant difference, compared to the control (One-way ANOVA).

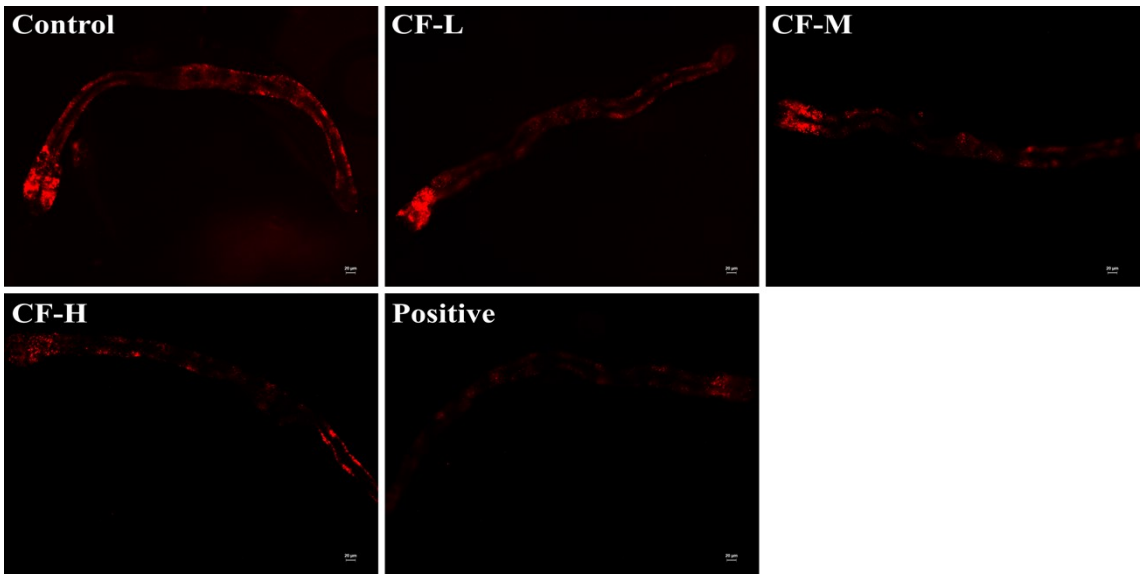

Fig. S3 Fluorescence images of Lipofuscin in *C. elegans*. Worms were cultured on NGM medium containing CF-L (20 μg/mL), CF-M (100 μg/mL) and CF-H (200 μg/mL), with no treatment as a blank control and treated with resveratrol (100 μM) was a positive control.

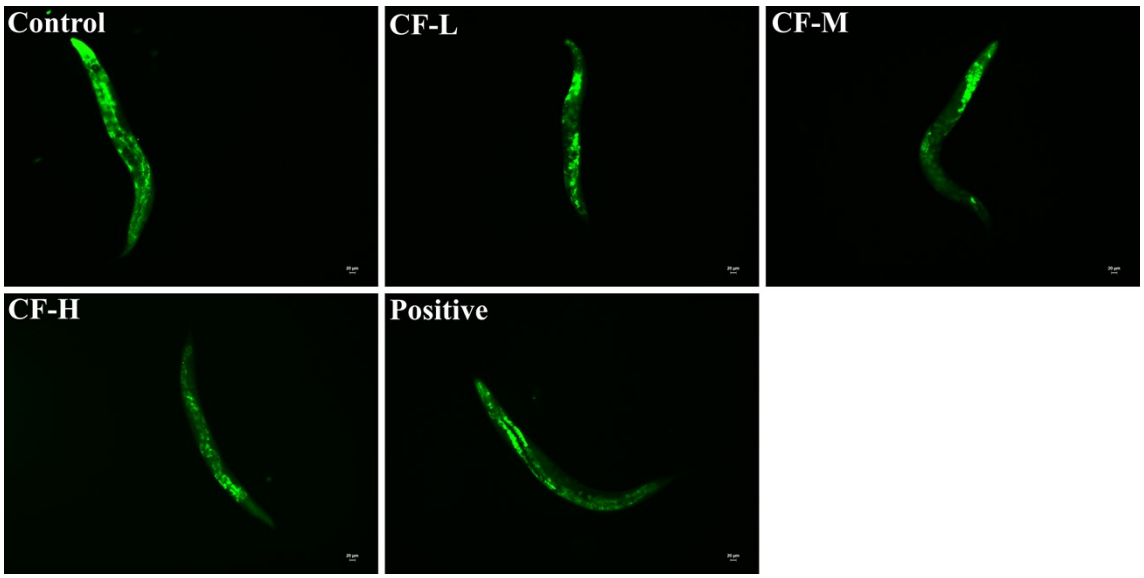

Fig. S4 Fluorescent images of ROS in *C. elegans*. Worms were cultured on NGM medium containing CF-L (20 μg/mL), CF-M (100 μg/mL) and CF-H (200 μg/mL), with no treatment as a blank control and treated with resveratrol (100 μM) was a positive control.

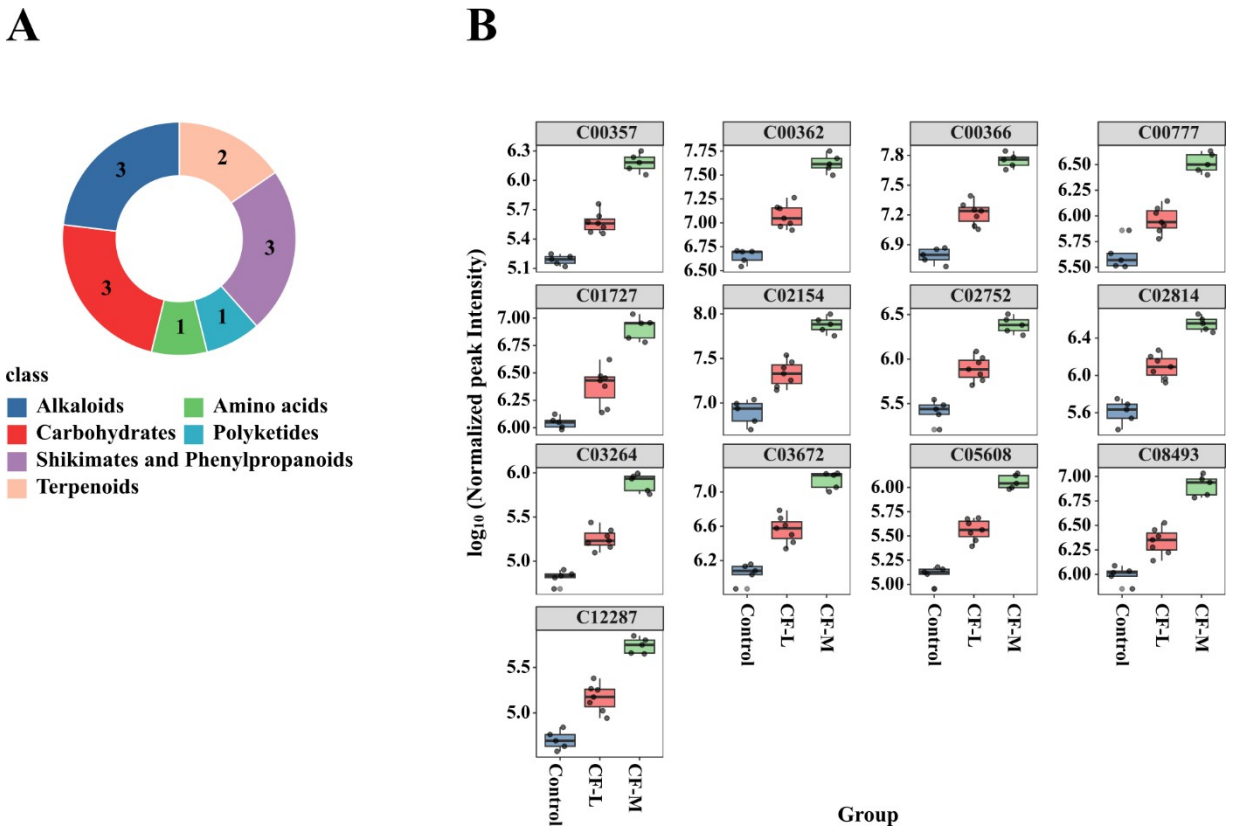

Fig. S5 Classes and abundance changes of key DMs after CF treatment. (A) The classification of differentially regulated metabolites. (B) The relative abundance (mean ± SD) of key differentially regulated metabolites in the control, CF-L and CF-M groups. The study included 5-7 replicates, and the worms were cultured on NGM medium supplemented with CF-L (20 μg/mL) and CF-M (100 μg/mL). No treatment was used as the control.