

# **Well-tolerated Spirulina extract inhibits influenza virus replication and reduces virus-induced mortality**

**Yi-Hsiang Chen<sup>1,3</sup>, Gi-Kung Chang<sup>1</sup>, Shu-Ming Kuo<sup>1</sup>, Sheng-Yu Huang<sup>1,3</sup>,  
I-Chen Hu<sup>5</sup>, Yu-Lun Lo<sup>1</sup>, and Shin-Ru Shih<sup>1,2,3,4,\*</sup>**

<sup>1</sup>Research Centre for Emerging Viral Infections,

<sup>2</sup>Department of Medical Biotechnology and Laboratory Science, and

<sup>3</sup>Graduate Institute of Biomedical Sciences, College of Medicine, Chang Gung University,

Taoyuan, Taiwan, ROC.

<sup>4</sup>Clinical Virology Lab, Department of Clinical Pathology, Chang Gung Memorial Hospital,

Taoyuan, Taiwan, ROC.

<sup>5</sup>Institute of Bioinformatics and Structural Biology, National Tsing-Hua University, Hsinchu,

Taiwan, ROC.

\*Correspondence should be addressed to: S.R.S (email: srshih@mail.cgu.edu.tw)

## Supplementary Information

Supplementary Table S1. Hematology results in rats (Mean  $\pm$  SD, N=10)

Parameters	Sex	Dose (mg/kg/day)			
		0	750	1500	3000
WBC ( $10^3/\mu\text{L}$ )	Male	11.058 $\pm$ 3.260	11.769 $\pm$ 3.089	14.721 $\pm$ 3.362	13.434 $\pm$ 4.067
	Female	11.158 $\pm$ 3.107	11.104 $\pm$ 3.626	11.387 $\pm$ 2.467	11.280 $\pm$ 4.141
RBC ( $10^6/\mu\text{L}$ )	Male	7.867 $\pm$ 0.238	8.066 $\pm$ 0.456	7.889 $\pm$ 0.375	7.809 $\pm$ 0.236
	Female	8.447 $\pm$ 0.488	8.287 $\pm$ 0.585	8.558 $\pm$ 0.555	8.392 $\pm$ 0.517
Hb (g/dL)	Male	14.83 $\pm$ 0.52	15.22 $\pm$ 0.94	15.19 $\pm$ 0.57	15.00 $\pm$ 0.57
	Female	15.64 $\pm$ 0.91	15.47 $\pm$ 1.19	15.98 $\pm$ 0.78	15.69 $\pm$ 0.83
Ht (%)	Male	46.98 $\pm$ 1.41	48.19 $\pm$ 2.82	47.80 $\pm$ 1.75	47.14 $\pm$ 1.83
	Female	48.35 $\pm$ 2.94	47.64 $\pm$ 3.34	48.93 $\pm$ 2.34	48.23 $\pm$ 2.51
MCV (fl)	Male	59.66 $\pm$ 0.88	59.76 $\pm$ 1.13	60.58 $\pm$ 1.01	60.37 $\pm$ 1.40
	Female	57.24 $\pm$ 1.60	57.52 $\pm$ 0.88	57.24 $\pm$ 1.75	57.52 $\pm$ 1.63
MCH (pg)	Male	18.86 $\pm$ 0.40	18.87 $\pm$ 0.41	19.26 $\pm$ 0.27	19.22 $\pm$ 0.47
	Female	18.49 $\pm$ 0.50	18.68 $\pm$ 0.33	18.67 $\pm$ 0.68	18.71 $\pm$ 0.46
MCHC (%)	Male	31.59 $\pm$ 0.46	31.58 $\pm$ 0.27	31.81 $\pm$ 0.41	31.81 $\pm$ 0.38
	Female	32.32 $\pm$ 0.37	32.45 $\pm$ 0.46	32.63 $\pm$ 0.64	32.53 $\pm$ 0.46
Platelet ( $10^3/\mu\text{L}$ )	Male	1134.0 $\pm$ 155.0	1152.4 $\pm$ 156.4	1088.8 $\pm$ 168.8	1150.2 $\pm$ 147.8
	Female	1219.0 $\pm$ 86.0	1197.0 $\pm$ 164.8	1244.3 $\pm$ 104.5	1209.2 $\pm$ 106.5

\*: P < 0.05

Supplementary Table S2. Serum Chemistry in rats (Mean  $\pm$  SD, N=10)

Parameters	Sex	Dose (mg/kg/day)			
		0	750	1500	3000
AST (U/L)	Male	128.2 $\pm$ 40.8	101.3 $\pm$ 10.3*	104.7 $\pm$ 18.3	102.1 $\pm$ 12.9*
	Female	105.0 $\pm$ 51.5	87.5 $\pm$ 8.2*	90.0 $\pm$ 21.1	94.2 $\pm$ 16.5
ALT (U/L)	Male	34.0 $\pm$ 4.1	31.5 $\pm$ 5.1	30.2 $\pm$ 4.2	27.9 $\pm$ 4.7
	Female	34.8 $\pm$ 38.7	23.8 $\pm$ 3.1	23.8 $\pm$ 5.7	26.5 $\pm$ 5.9
ALP (U/L)	Male	193.6 $\pm$ 25.4	169.0 $\pm$ 17.5	169.6 $\pm$ 26.8	164.6 $\pm$ 38.1
	Female	100.1 $\pm$ 21.8	94.1 $\pm$ 22.3	98.0 $\pm$ 28.1	94.1 $\pm$ 19.9
Total proteins (g/dL)	Male	6.59 $\pm$ 0.21	6.52 $\pm$ 0.36	6.47 $\pm$ 0.31	6.56 $\pm$ 0.35
	Female	7.14 $\pm$ 0.54	7.28 $\pm$ 0.30	7.37 $\pm$ 0.32	7.16 $\pm$ 0.24
Uric acid (mg/dL)	Male	2.67 $\pm$ 0.59	2.71 $\pm$ 0.53	2.68 $\pm$ 0.56	2.89 $\pm$ 1.02
	Female	2.44 $\pm$ 0.62	2.87 $\pm$ 0.50	2.35 $\pm$ 0.34	2.36 $\pm$ 0.45
$\gamma$ GT	Male	0.867 $\pm$ 0.454	0.767 $\pm$ 0.565	0.713 $\pm$ 0.635	0.506 $\pm$ 0.434
	Female	0.651 $\pm$ 0.644	1.083 $\pm$ 0.749	0.681 $\pm$ 0.500	0.785 $\pm$ 0.608
Glucose (mg/dL)	Male	146.4 $\pm$ 26.9	149.2 $\pm$ 24.8	155.5 $\pm$ 24.7	165.6 $\pm$ 43.4
	Female	143.4 $\pm$ 37.6	162.5 $\pm$ 38.3.	142.2 $\pm$ 34.6	144.8 $\pm$ 22.5
Creatinine (mg/dL)	Male	0.41 $\pm$ 0.06	0.41 $\pm$ 0.03	0.42 $\pm$ 0.04	0.43 $\pm$ 0.05
	Female	0.50 $\pm$ 0.05	0.47 $\pm$ 0.05	0.49 $\pm$ 0.07	0.47 $\pm$ 0.07

\*: P&lt;0.05

Supplementary Table S3. Composition analysis of Spirulina extract

	Content~% (w/w)
Protein	39.33 ± 5.6
Polysaccharide	11.79 ± 5.7
Nucleic acid	19.29 ± 2.7
Water	5 ± 1
Ash	1.2 ± 0.3
Others	23.39

Protein content determined by Bradford assay

Polysaccharide content determined by the phenol-sulfuric acid method

Nucleotide content determined by OD260 absorbance

Water content determined by the loss on drying method

Ash content determined by incineration at 600°C for 7 hrs.

---

Supplementary Table S4. Antiviral activity of the further fractionation of Spirulina extract against Flu A/WSN/33 virus

---

	<b>EC50 (mg/mL)</b>
Spirulina extract	0.955 ± 0.052
Spirulina extract (> 100KD)	0.657 ± 0.202
Spirulina extract (< 100KD)	Not detected
Spirulina extract (Ethanol prec.)	0.602 ± 0.04
Spirulina extract (DEAE)	0.668 ± 0.033
Spirulina extract (TCA)	0.835 ± 0.02
Spirulina extract (HWE)	Not detected
C-phycoerythrin	Not detected
Allylphycoerythrin	Not detected

---

>100KD and <100KD: Fractionized with molecular size at 100KD by “Amicon Ultra-15 Centrifugal Filter Unit with Ultracel-100 membrane;

Ethanol prec.: Fractionized with 70% ethanol precipitation;

DEAE: Fractionized with DEAE gel (Sepharose™ Fast Flow) for negative charge molecules;

TCA: 10% Trichloroacetic acid precipitation to remove protein fraction;

HWE: Hot water extraction with 99°C for 1hr. to denature proteins fraction;

EC50: 50% effective concentration, based on neutralization test;

All data represent mean ± SD for three independent experiments.