Supplementary Materials
Suk-SaiYasna Remedy, A Traditional Thai Medicine, Mitigates Stress-Induced Cognitive Impairment via Keap1-Nrf2 Pathway

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1. ***Statistical analysis of SSY on Unpredictable Chronic Mild Stress (UCMS)-Induced Cognitive Impairment.***

Table S1. Pair Student' s *t*-test and One-way analysis of variance (ANOVA) test of escape latency time (training phase session) on Morris Water Maze (MWM) test

Group comparison	ANOVA followed by Tukey's post hoc test	
	P	***F*(DF$_{between\ group}$, DF$_{residual}$)**
Day 2		
non-stress group VS. UCMS + vehicle group	< 0.001	$t(22) = -4.820$
UMCS + vehicle group vs. UCMS + Vitamin E group	Not significant	$F(4,55) = 4.183$
UMCS + vehicle group vs. UCMS + SSY20 group	Not significant	
UMCS + vehicle group vs. UCMS + SSY100 group	Not significant	
UMCS + vehicle group vs. UCMS + SSY500 group	Not significant	
Day 3		
non-stress group VS. UCMS + vehicle group	< 0.001	$t(22) = -7.783$
UMCS + vehicle group vs. UCMS + Vitamin E group	< 0.001	$F(4,55) = 13.026$
UMCS + vehicle group vs. UCMS + SSY20 group	Not significant	
UMCS + vehicle group vs. UCMS + SSY100 group	< 0.05	
UMCS + vehicle group vs. UCMS + SSY500 group	< 0.001	
Day 4		
non-stress group VS. UCMS + vehicle group	< 0.001	$t(22) = -7.422$
UMCS + vehicle group vs. UCMS + Vitamin E group	< 0.001	$F(4,55) = 21.031$
UMCS + vehicle group vs. UCMS + SSY20 group	Not significant	
UMCS + vehicle group vs. UCMS + SSY100 group	< 0.001	
UMCS + vehicle group vs. UCMS + SSY500 group	< 0.001	
Day 5		
non-stress group VS. UCMS + vehicle group	< 0.001	$t(22) = -10.101$
UMCS + vehicle group vs. UCMS + Vitamin E group	< 0.001	$F(4,55) = 12.177$
UMCS + vehicle group vs. UCMS + SSY20 group	Not significant	
UMCS + vehicle group vs. UCMS + SSY100 group	< 0.001	
UMCS + vehicle group vs. UCMS + SSY500 group	< 0.001	

Table S2. Pair Student' s *t*-test and One-way analysis of variance (ANOVA) test of time in target quadrant (probe test session) on MWM test

Group comparison	ANOVA followed by Tukey's post hoc test	
	P	***F*(DF$_{between\ group}$, DF$_{residual}$)**
non-stress group VS. UCMS + vehicle group	< 0.001	$t(22) = 9.745$
UMCS + vehicle group vs. UCMS + Vitamin E group	< 0.001	$F(4,55) = 6.970$
UMCS + vehicle group vs. UCMS + SSY20 group	Not significant	
UMCS + vehicle group vs. UCMS + SSY100 group	< 0.05	
UMCS + vehicle group vs. UCMS + SSY500 group	< 0.001	
UCMS + SSY20 group vs. UCMS + SSY500 group	< 0.05	

Table S3. Pair Student' s *t*-test and One-way analysis of variance (ANOVA) test of % spontaneous alternation on Y-maze test

Group comparison	ANOVA followed by Tukey's post hoc test	
	P	***F*(DF$_{between\ group}$, DF$_{residual}$)**
non-stress group VS. UCMS + vehicle group	< 0.001	$t(22) = 10.885$
UMCS + vehicle group vs. UCMS + Vitamin E group	< 0.001	$F(4,55) = 21.045$
UMCS + vehicle group vs. UCMS + SSY20 group	Not significant	
UMCS + vehicle group vs. UCMS + SSY100 group	< 0.001	
UMCS + vehicle group vs. UCMS + SSY500 group	< 0.001	
UCMS + SSY20 group vs. UCMS + SSY100 group	Not significant	
UCMS + SSY20 group vs. UCMS + SSY500 group	< 0.001	
UCMS + SSY100 group vs. UCMS + SSY500 group	0.006	

Table S4. Pair Student' s *t*-test and One-way analysis of variance (ANOVA) test of % discrimination index on Novel Object Recognition Test (NORT)

Group comparison	ANOVA followed by Tukey's post hoc test	
	P	***F*(DF$_{between\ group}$, DF$_{residual}$)**
non-stress group VS. UCMS + vehicle group	< 0.001	$t(22) = 15.180$
UMCS + vehicle group vs. UCMS + Vitamin E group	< 0.001	$F(4,55) = 7.664$
UMCS + vehicle group vs. UCMS + SSY20 group	Not significant	
UMCS + vehicle group vs. UCMS + SSY100 group	< 0.001	
UMCS + vehicle group vs. UCMS + SSY500 group	< 0.001	
UCMS + SSY20 group vs. UCMS + SSY100 group	Not significant	
UCMS + SSY20 group vs. UCMS + SSY500 group	0.048	
UCMS + SSY100 group vs. UCMS + SSY500 group	Not significant	

2. ***Statistical analysis of the SSY extract on UCMS-Induced hypersecretion of lipid peroxidation, Superoxide dismutase (SOD) (B) and Catalase (CAT)***

Table S5. Pair Student' s *t*-test and One-way analysis of variance (ANOVA) test of concentration of MDA (nmol/mg protein) in lipid peroxidation test

Frontal cortex		
Group comparison	**ANOVA followed by Tukey's post hoc test**	
	P	***F*(DF$_{between\ group}$, DF$_{residual}$)**
non-stress group VS. UCMS + vehicle group	< 0.001	$t(8) = -6.102$
UMCS + vehicle group vs. UCMS + Vitamin E group	< 0.001	$F(4,20) = 5.162$
UMCS + vehicle group vs. UCMS + SSY20 group	Not significant	
UMCS + vehicle group vs. UCMS + SSY100 group	0.006	
UMCS + vehicle group vs. UCMS + SSY500 group	< 0.001	
UCMS + SSY20 group vs. UCMS + SSY100 group	Not significant	
UCMS + SSY20 group vs. UCMS + SSY500 group	0.040	
UCMS + SSY100 group vs. UCMS + SSY500 group	Not significant	
Hippocampus		
non-stress group VS. UCMS + vehicle group	< 0.001	$t(8) = -4.832$
UMCS + vehicle group vs. UCMS + Vitamin E group	< 0.001	$F(4,20) = 3.376$
UMCS + vehicle group vs. UCMS + SSY20 group	Not significant	
UMCS + vehicle group vs. UCMS + SSY100 group	0.038	
UMCS + vehicle group vs. UCMS + SSY500 group	< 0.001	
UCMS + SSY20 group vs. UCMS + SSY100 group	Not significant	
UCMS + SSY20 group vs. UCMS + SSY500 group	0.033	
UCMS + SSY100 group vs. UCMS + SSY500 group	Not significant	

Table S6. Pair Student' s *t*-test and One-way analysis of variance (ANOVA) test of SOD activity (U/mg protein) in SOD test

Frontal cortex		
Group comparison	**ANOVA followed by Tukey's post hoc test**	
	P	***F*(DF$_{between\ group}$, DF$_{residual}$)**
non-stress group VS. UCMS + vehicle group	< 0.001	$t(8) = 7.875$
UMCS + vehicle group vs. UCMS + Vitamin E group	< 0.001	$F(4,20) = 18.332$
UMCS + vehicle group vs. UCMS + SSY20 group	Not significant	
UMCS + vehicle group vs. UCMS + SSY100 group	0.048	
UMCS + vehicle group vs. UCMS + SSY500 group	< 0.001	
UCMS + SSY20 group vs. UCMS + SSY100 group	0.034	
UCMS + SSY20 group vs. UCMS + SSY500 group	0.005	
UCMS + SSY100 group vs. UCMS + SSY500 group	Not significant	
Hippocampus		
non-stress group VS. UCMS + vehicle group	< 0.001	$t(8) = 8.279$
UMCS + vehicle group vs. UCMS + Vitamin E group	< 0.001	$F(4,20) = 21.167$
UMCS + vehicle group vs. UCMS + SSY20 group	Not significant	
UMCS + vehicle group vs. UCMS + SSY100 group	0.021	
UMCS + vehicle group vs. UCMS + SSY500 group	< 0.001	
UCMS + SSY20 group vs. UCMS + SSY100 group	Not significant	
UCMS + SSY20 group vs. UCMS + SSY500 group	0.029	
UCMS + SSY100 group vs. UCMS + SSY500 group	Not significant	

Table S7. Pair Student' s *t*-test and One-way analysis of variance (ANOVA) test of CAT activity (U/mg protein) in CAT test

Frontal cortex		
Group comparison	**ANOVA followed by Tukey's post hoc test**	
	P	***F*(DF$_{between\ group}$, DF$_{residual}$)**
non-stress group VS. UCMS + vehicle group	< 0.001	$t(8) = 7.879$
UMCS + vehicle group vs. UCMS + Vitamin E group	< 0.001	$F(4,20) = 46.629$
UMCS + vehicle group vs. UCMS + SSY20 group	Not significant	
UMCS + vehicle group vs. UCMS + SSY100 group	< 0.001	
UMCS + vehicle group vs. UCMS + SSY500 group	< 0.001	
UCMS + SSY100 group vs. UCMS + SSY500 group	0.036	
UCMS + SSY20 group vs. UCMS + SSY500 group	< 0.001	
UCMS + SSY20 group vs. UCMS + SSY100 group	Not significant	
Hippocampus		
non-stress group VS. UCMS + vehicle group	< 0.001	$t(8) = 6.860$
UMCS + vehicle group vs. UCMS + Vitamin E group	< 0.001	$F(4,20) = 52.970$
UMCS + vehicle group vs. UCMS + SSY20 group	Not significant	
UMCS + vehicle group vs. UCMS + SSY100 group	< 0.001	
UMCS + vehicle group vs. UCMS + SSY500 group	< 0.001	
UCMS + SSY100 group vs. UCMS + SSY500 group	0.045	
UCMS + SSY20 group vs. UCMS + SSY500 group	< 0.001	
UCMS + SSY20 group vs. UCMS + SSY100 group	Not significant	

3. ***Statistical Analysis of Effect of the SSY on UCMS-Induced Impaired Antioxidant enzyme Frontal Cortex and Hippocampus Using Quantitative real-time polymerase chain reaction (qPCR)***

Table S8. Paired Student's *t*-test and One-way analysis of variance (ANOVA) test of Keap1 in frontal cortex and hippocampus

Frontal cortex		
Group comparison	**ANOVA followed by Tukey's post hoc test**	
	P	***F*(DF$_{between\ group}$, DF$_{residual}$)**
non-stress group VS. UCMS + vehicle group	< 0.001	$t(8) = -25.280$
UMCS + vehicle group vs. UCMS + Vitamin E group	< 0.001	$F(4,20) = 37.125$
UMCS + vehicle group vs. UCMS + SSY20 group	Not significant	
UMCS + vehicle group vs. UCMS + SSY100 group	< 0.001	
UMCS + vehicle group vs. UCMS + SSY500 group	< 0.001	
UCMS + SSY100 group vs. UCMS + SSY500 group	0.021	
UCMS + SSY20 group vs. UCMS + SSY500 group	< 0.001	
UCMS + SSY20 group vs. UCMS + SSY100 group	0.001	
Hippocampus		
non-stress group VS. UCMS + vehicle group	< 0.001	$t(8) = -22.411$
UMCS + vehicle group vs. UCMS + Vitamin E group	< 0.001	$F(4,20) = 107.041$
UMCS + vehicle group vs. UCMS + SSY20 group	Not significant	
UMCS + vehicle group vs. UCMS + SSY100 group	< 0.001	
UMCS + vehicle group vs. UCMS + SSY500 group	< 0.001	
UCMS + SSY100 group vs. UCMS + SSY500 group	0.041	
UCMS + SSY20 group vs. UCMS + SSY500 group	< 0.001	
UCMS + SSY20 group vs. UCMS + SSY500 group	0.002	

Table S9. Paired Student's *t*-test and One-way analysis of variance (ANOVA) test of nuclear factor erythroid 2-related factor 2 (Nrf2) in frontal cortex and hippocampus

Frontal cortex		
Group comparison	**ANOVA followed by Tukey's post hoc test**	
	P	***F*($DF_{between\ group}$, $DF_{residual}$)**
non-stress group VS. UCMS + vehicle group	< 0.001	$t(8) = 55.337$
UMCS + vehicle group vs. UCMS + Vitamin E group	< 0.001	$F(4,20) = 50.343$
UMCS + vehicle group vs. UCMS + SSY20 group	Not significant	
UMCS + vehicle group vs. UCMS + SSY100 group	< 0.001	
UMCS + vehicle group vs. UCMS + SSY500 group	< 0.001	
UCMS + SSY20 group vs. UCMS + SSY500 group	0.039	
UCMS + SSY100 group vs. UCMS + SSY500 group	Not significant	
UCMS + SSY20 group vs. UCMS + SSY100 group	0.011	
Hippocampus		
non-stress group VS. UCMS + vehicle group	< 0.001	$t(8) = 35.777$
UMCS + vehicle group vs. UCMS + Vitamin E group	< 0.001	$F(4,20) = 70.092$
UMCS + vehicle group vs. UCMS + SSY20 group	Not significant	
UMCS + vehicle group vs. UCMS + SSY100 group	< 0.001	
UMCS + vehicle group vs. UCMS + SSY500 group	< 0.001	
UCMS + SSY20 group vs. UCMS + SSY500 group	0.037	
UCMS + SSY20 group vs. UCMS + SSY500 group	Not significant	
UCMS + SSY20 group vs. UCMS + SSY100 group	Not significant	

Table S10. Paired Student's *t*-test and One-way analysis of variance (ANOVA) test of heme oxygenase-1 (HO-1) in frontal cortex and hippocampus

Frontal cortex		
Group comparison	**ANOVA followed by Tukey's post hoc test**	
	P	***F*($DF_{between\ group}$, $DF_{residual}$)**
non-stress group VS. UCMS + vehicle group	< 0.001	$t(8) = 13.809$
UMCS + vehicle group vs. UCMS + Vitamin E group	< 0.001	$F(4,20) = 51.588$
UMCS + vehicle group vs. UCMS + SSY20 group	Not significant	
UMCS + vehicle group vs. UCMS + SSY100 group	< 0.001	
UMCS + vehicle group vs. UCMS + SSY500 group	< 0.001	
UCMS + SSY20 group vs. UCMS + SSY500 group	< 0.001	
UCMS + SSY100 group vs. UCMS + SSY500 group	< 0.001	
Hippocampus		
non-stress group VS. UCMS + vehicle group	< 0.001	$t(8) = 45.882$
UMCS + vehicle group vs. UCMS + Vitamin E group	< 0.001	$F(4,20) = 54.644$
UMCS + vehicle group vs. UCMS + SSY20 group	Not significant	
UMCS + vehicle group vs. UCMS + SSY100 group	< 0.001	
UMCS + vehicle group vs. UCMS + SSY500 group	< 0.001	
UCMS + SSY100 group vs. UCMS + SSY500 group	< 0.001	
UCMS + SSY20 group vs. UCMS + SSY100 group	< 0.001	
UCMS + SSY20 group vs. UCMS + SSY500 group	< 0.001	

Table S11. Paired Student's *t*-test and One-way analysis of variance (ANOVA) test of NAD(P)H quinone dehydrogenase 1 (NQO1) in frontal cortex and hippocampus

Frontal cortex		
Group comparison	**ANOVA followed by Tukey's post hoc test**	
	P	***F*(DF$_{between\ group}$, DF$_{residual}$)**
non-stress group VS. UCMS + vehicle group	< 0.001	*t*(8) = -10.176
UMCS + vehicle group vs. UCMS + Vitamin E group	< 0.001	*F*(4,20) = 95.769
UMCS + vehicle group vs. UCMS + SSY20 group	Not significant	
UMCS + vehicle group vs. UCMS + SSY100 group	< 0.001	
UMCS + vehicle group vs. UCMS + SSY500 group	< 0.001	
UMCS + SSY20 group vs. UCMS + SSY100 group	Not significant	
UCMS + SSY20 group vs. UCMS + SSY500 group	< 0.001	
UCMS + SSY100 group vs. UCMS + SSY500 group	< 0.001	
Hippocampus		
non-stress group VS. UCMS + vehicle group	< 0.001	*t*(8) = -17.656
UMCS + vehicle group vs. UCMS + Vitamin E group	< 0.001	*F*(4,20) = 56.235
UMCS + vehicle group vs. UCMS + SSY20 group	Not significant	
UMCS + vehicle group vs. UCMS + SSY100 group	< 0.001	
UMCS + vehicle group vs. UCMS + SSY500 group	< 0.001	
UMCS + SSY20 group vs. UCMS + SSY100 group	0.026	
UMCS + SSY100 group vs. UCMS + SSY500 group	< 0.001	
UMCS + SSY20 group vs. UCMS + SSY500 group	< 0.001	

Table S12. Validation results of the LC-MS/MS method.

Chemical constituent	Retention time (min)	ION precursor/product	MS Mode	Standard curve equations	R^2	LOD (μg/mg)	LOQ (μg/mg)
CBD	10.50	315.3/193.197, 259.238	ESI positive	y = 737.57x + 17.350	0.9960	0.03	0.11
delta-9-THC	12.10	315.3/193.197, 259.238	ESI positive	y = 1101.7x + 145.90	0.9920	0.07	0.22
delta-8-THC	12.15	315.3/193.197, 259.238	ESI positive	y = 816.87x + 69.338	0.9932	0.04	0.12
THCA-A	12.75	357.2/313.28, 245.137	ESI negative	y = 71.227x + 29.183	0.9949	0.15	0.49

Table S13. Validation results of the analytical method for determination of piperine, gingerol, gallic acid, myricetin, myristicin, thymoquinone, cinnamic acid, costunolide, and azadirachtin content in the SSY extract

Chemical constituent	Linearity	Range (μg/ml)	Accuracy (%Recovery)	Precision Repeatability (%RSD)	Precision Intermediate Precision (%RSD)	LOD (S/N)	LOQ (S/N)	UV Purity	Robustness (Flowrate) (%RSD)
Piperine	y = 272.52x - 110.36 R^2 = 0.9960	1-8	95.55-98.33	0.04-0.54	0.05-1.11	0.1 (5.98)	0.2 (12.70)	999.96	0.4, 0.5, 0.6 (0.64-2.77)
6-Gingerol	y = 134.14x - 43.639 R^2 = 0.9988	1-10	96.34-98.09	0.05-1.33	0.03-1.20	0.1 (14.97)	0.2 (20.78)	999.94	0.4, 0.5, 0.6 (0.22-0.58)
Gallic acid	y = 134.14x - 43.639 R^2 = 0.9988	1-8	98.45-100.91	0.04-0.44	0.01-0.04	0.1 (20.34)	0.2 (20.36)	996.46	0.4, 0.5, 0.6 (0.16-0.18)
Myricetin	y = 97.20x - 12.502 R^2 = 0.9981	1-8	100.51-103.50	0.23-0.83	0.03-0.19	0.1 (17.30)	0.2 (13.05)	996.57	0.4, 0.5, 0.6 (0.08-0.52)
Myristicin	y = 419.38x – 134.70 R^2 = 0.999	1-10	98.10-104.56	0.04-0.64	0.04-0.65	0.1 (37.89)	0.2 (14.51)	999.84	0.4, 0.5, 0.6 (0.04-0.05)
Thymoquinone	y = 29.565x + 1.627 R^2 = 0.9977	1-10	99.81-101.55	0.30-0.77	0.02-1.20	0.1 (33.06)	0.2 (12.67)	999.99	0.4, 0.5, 0.6 (0.07-3.06)
Cinnamic acid	y = 266.83x + 63.244 R^2 = 0.9974	1-10	95.37-103.77	0.12-0.90	0.11-0.92	0.1 (21.96)	0.2 (28.24)	999.93	0.4, 0.5, 0.6 (0.20-0.30)
Costunolide	y = 161.74x + 5.0821 R^2 = 0.9967	1-10	90.04-103.05	0.16-0.84	0.07-0.87	0.1 (20.71)	0.2 (70.42)	999.89	0.4, 0.5, 0.6 (0.61-0.63)
Azadirachtin	y = 24.738x + 2.7889 R^2 = 0.9998	1-10	99.03-100.51	0.23-1.02	0.02-1.50	0.1 (34.59)	0.2 (15.39)	999.94	0.4, 0.5, 0.6 (0.43-0.39)
Acceptance criteria	R^2 = 0.99	-	80-115	%RSD ≤ 6	%RSD ≤ 6	S/N ≥ 3	S/N ≥ 10	-	%RSD ≤ 6

Figure S1. The linearity of y-axis representing Area (mAU*s) and the x-axis indicating concentration (microg/ml) for all reference standards

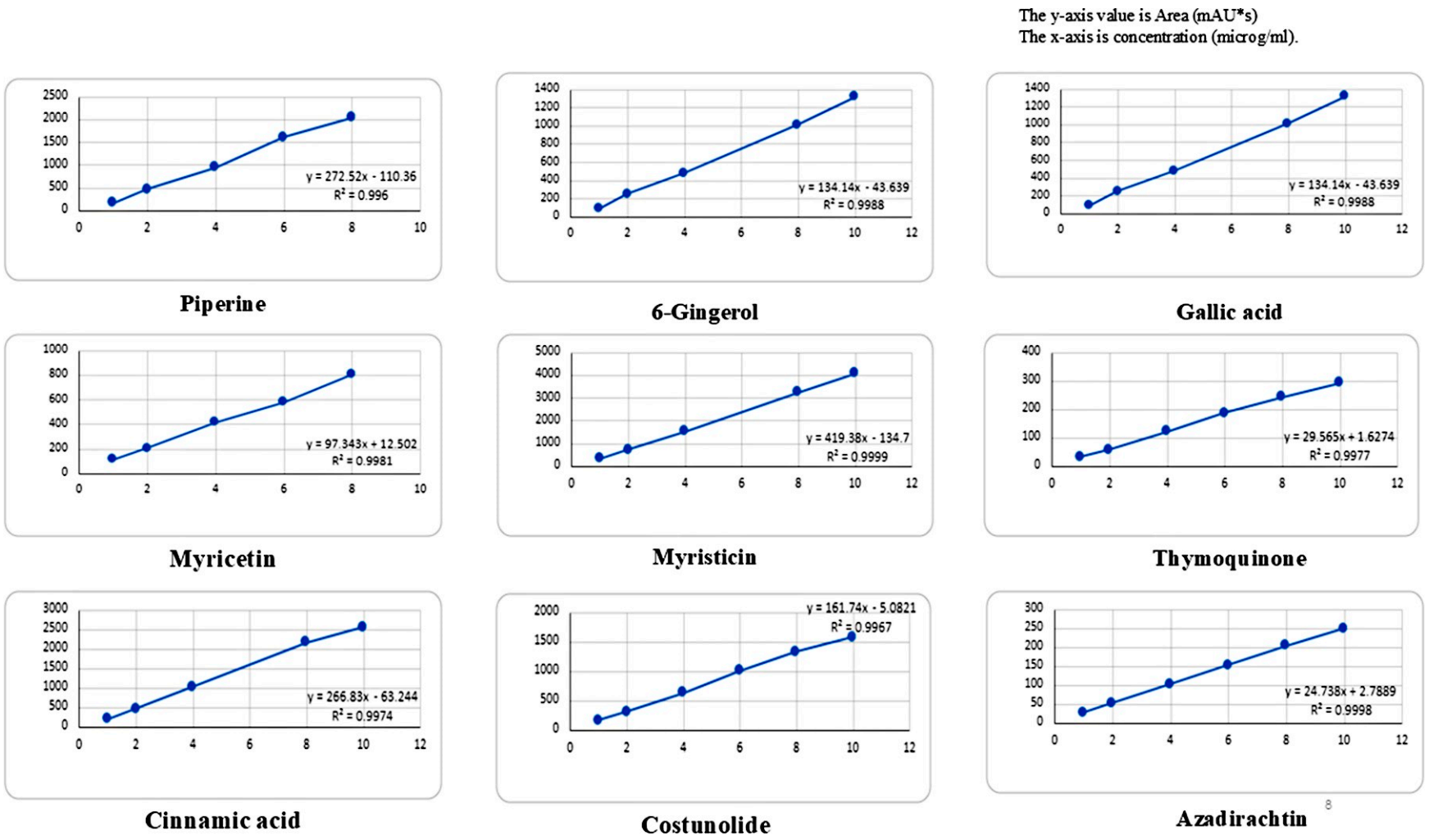


Table S14. One-week schedule of unpredictable chronic mild stresses protocol.

Activity/Days	Mon.	Tue.	Wed.	Thu.	Fri.	Sat.	Sun.
1. Deprivation of food and water (18 hrs.)	3:00 pm-9:00 am						
2. Sucrose preference test (1 hr.)		9:00 am-3:00 pm					
3. Tilted cage 45° (12, 12 hrs.)		08:00 pm-08:00 am				7:00 pm-7.00 am	
4. 5 micro pellets restricted access to food (1 hr.)		3:00-4:00 pm					
5. Empty bottle exposure (3, 3 hrs.)			2:00 pm-5:00 pm		9:00 am-12:00 am		
6. Wet cage 100 g sawdust bedding (21 hrs.)					1:00 pm-10:00 am		
7. exposure to light (36 hrs.)	(Sun.) 6:00 am-(Mon.) 6:00 pm		7:00 pm-07:00 am				(Sun.) 6:00 am-(Mon.) -6:00 pm
8. Sound is intermittent (3, 5 hrs.)			9:00 am-12:00 pm			12:00 pm-5:00 am	
9. Caging in pairs (2 hrs.)		5:00 pm-7:00 pm					1:00 pm-3:00 pm