

Appendix A Supplementary information

Phytochemical and Biological Investigation of *Jatropha pelargoniifolia* Root Native to the Kingdom of Saudi Arabia

Hanan Y. Aati¹, Ali A. El-Gamal^{1,2}, Oliver Kayser³

¹Department of Pharmacognosy, Faculty of Pharmacy, King Saud University, Riyadh 11451, Saudi Arabia

²Department of Pharmacognosy, College of Pharmacy, Mansoura University, El-Mansoura 35516, Egypt

³TU Dortmund University, Technical Biochemistry, Emil-Figge-Strasse 66, D-44227 Dortmund, Germany

Corresponding Author

Ali Ali El-Gamal

¹Department of Pharmacognosy, Faculty of Pharmacy, King Saud University, Riyadh 11451, Saudi Arabia. Phone +966-569780176.

E-mail: aelgamel@ksu.edu.sa

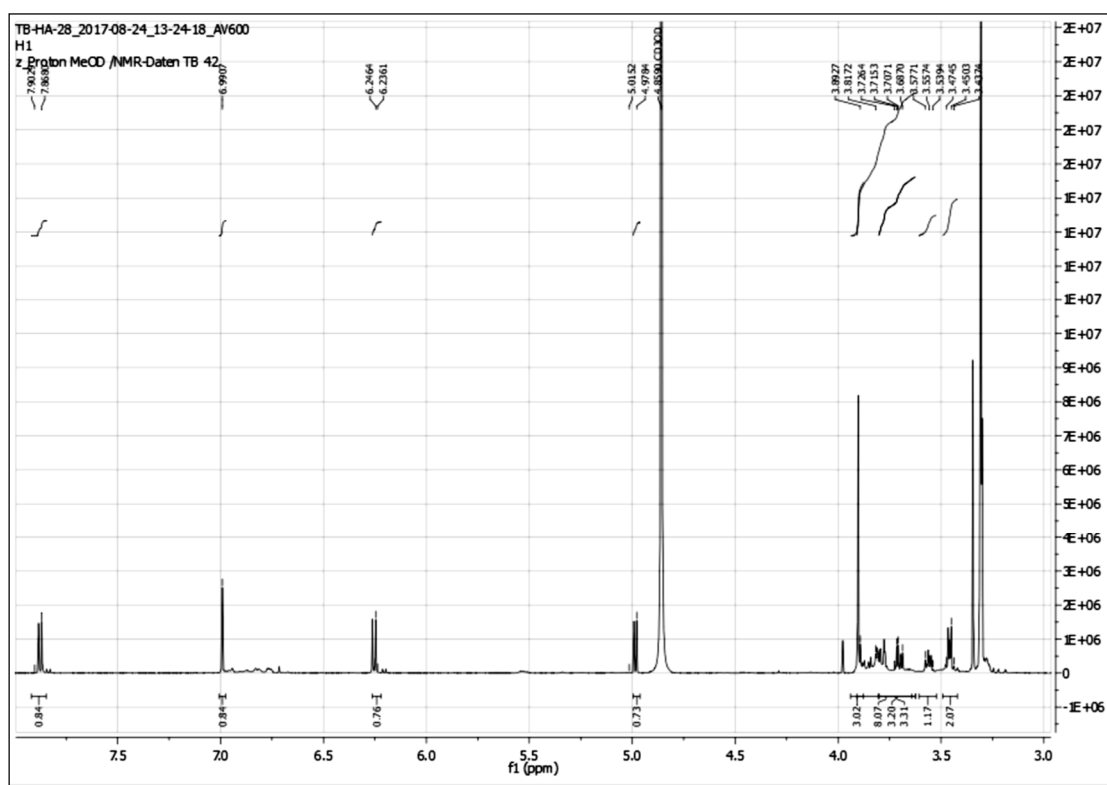


Figure S1: ^1H NMR spectrum for compound **15** in CD_3OD (600 MHz)

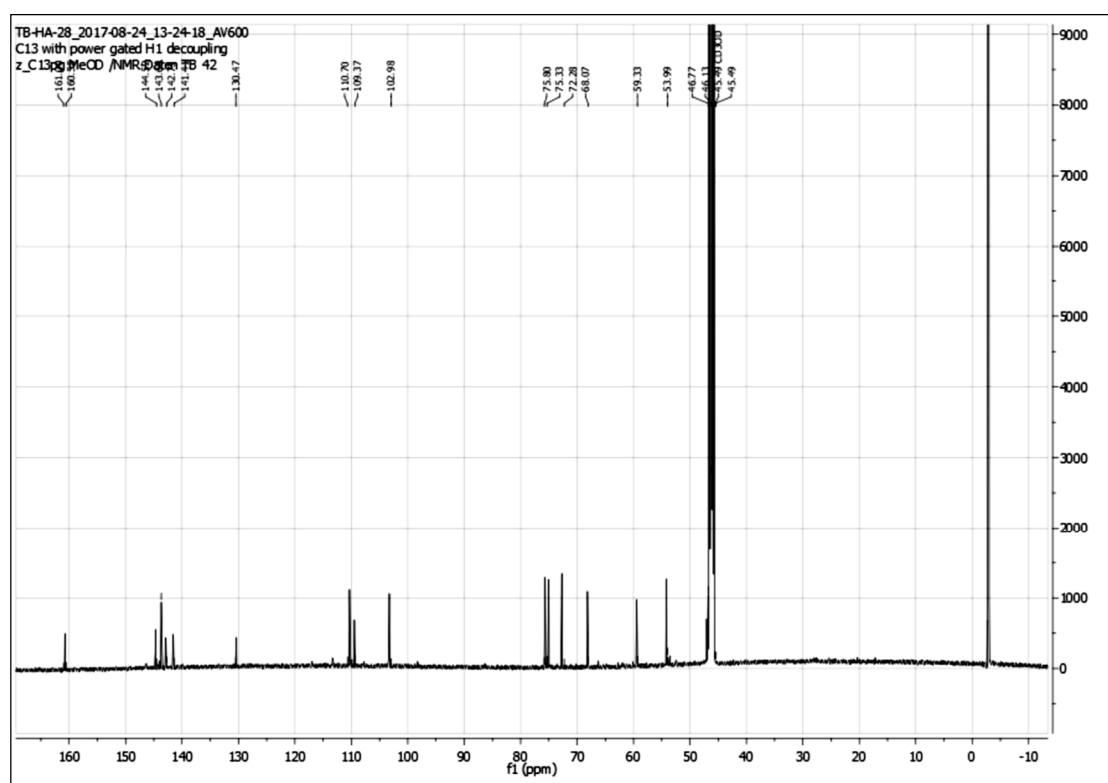
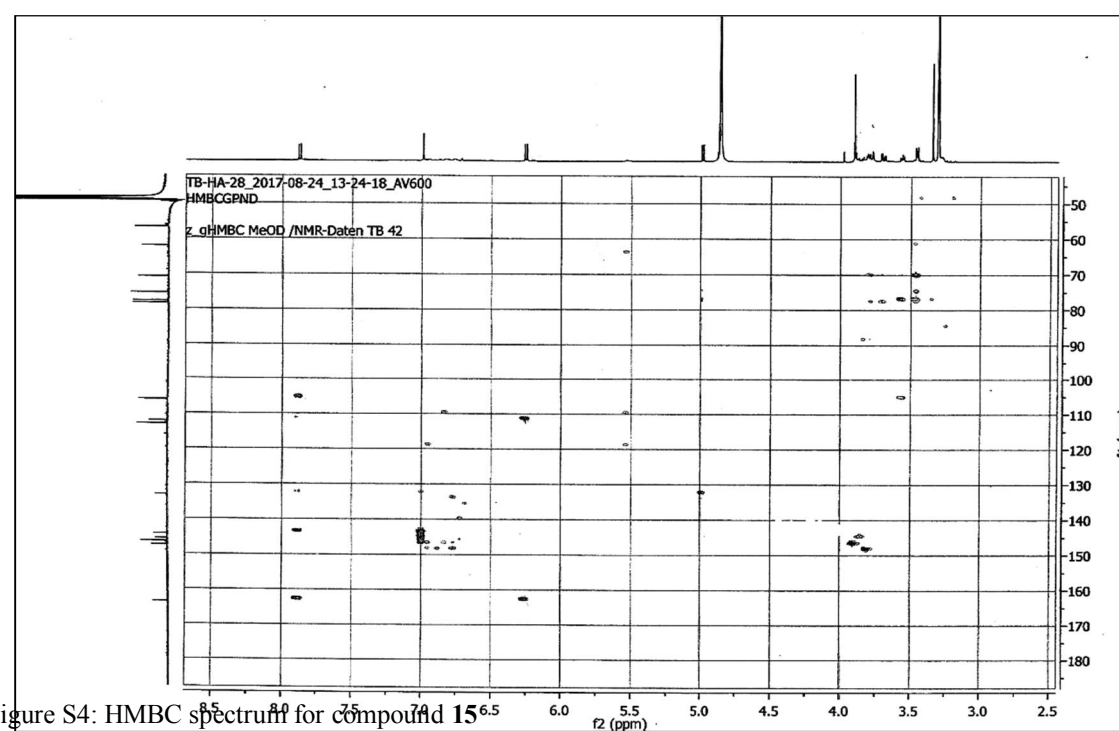
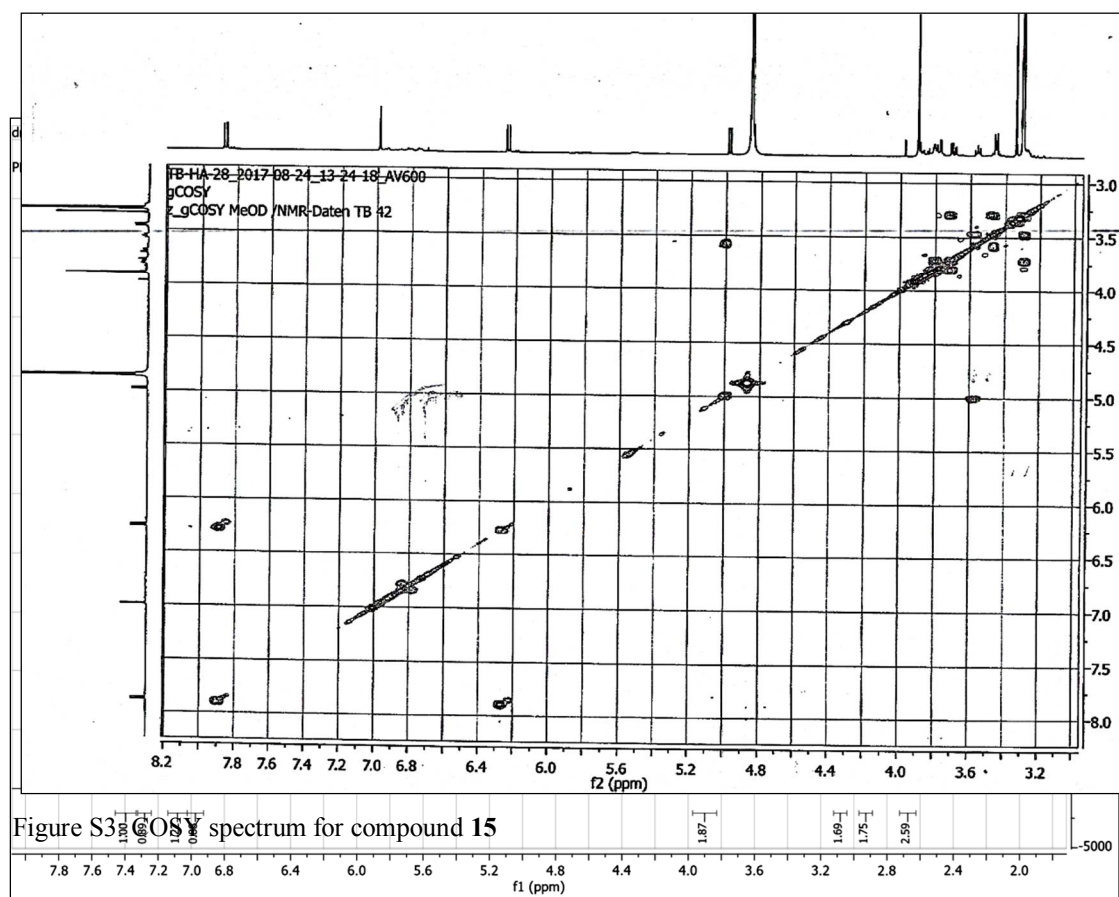


Figure S2: ^{13}C NMR spectrum for compound **15** in CD_3OD (125 MHz)



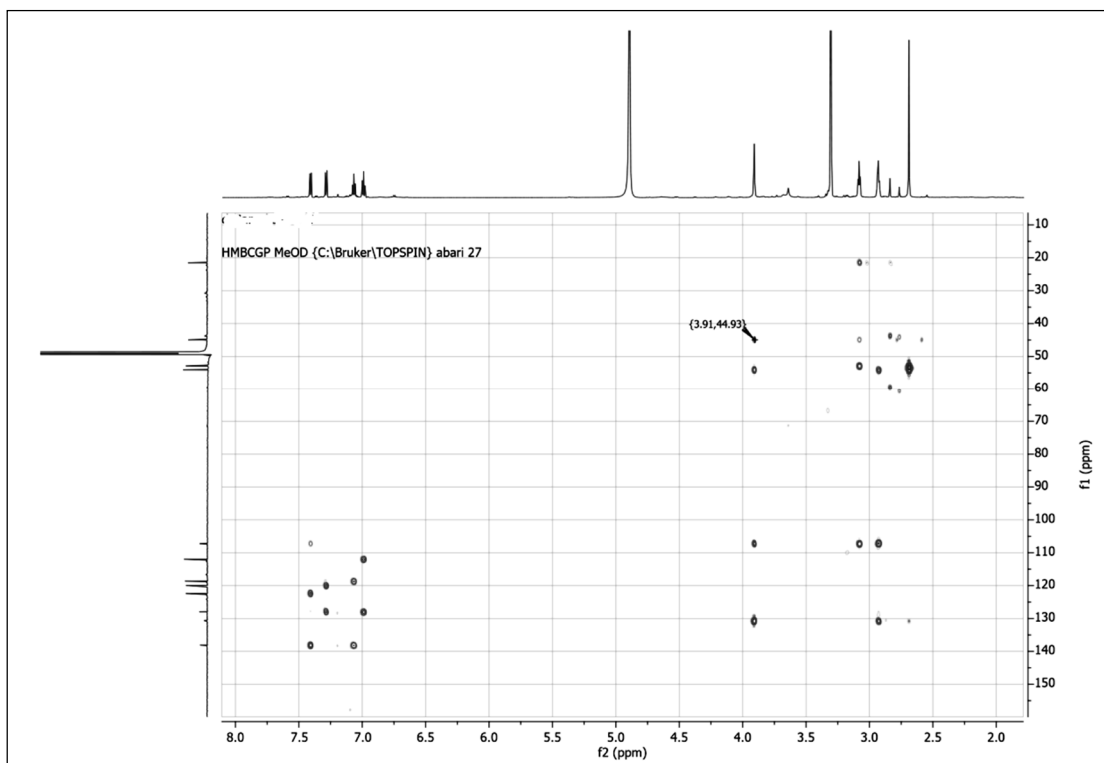


Figure S5: ^1H NMR spectrum for compound **18** in CD_3OD (700 MHz)

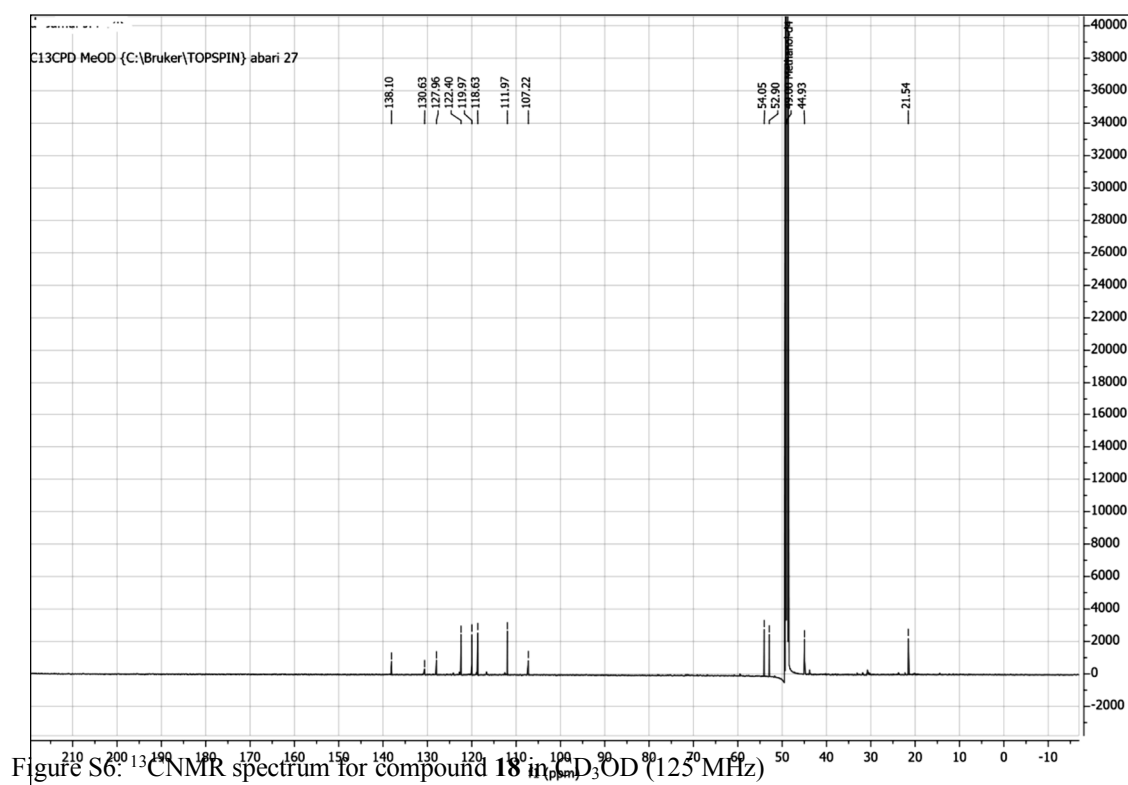


Figure S6: ^{13}C NMR spectrum for compound **18** in CD_3OD (125 MHz)

Figure S7: HMBC spectrum for compound **18**

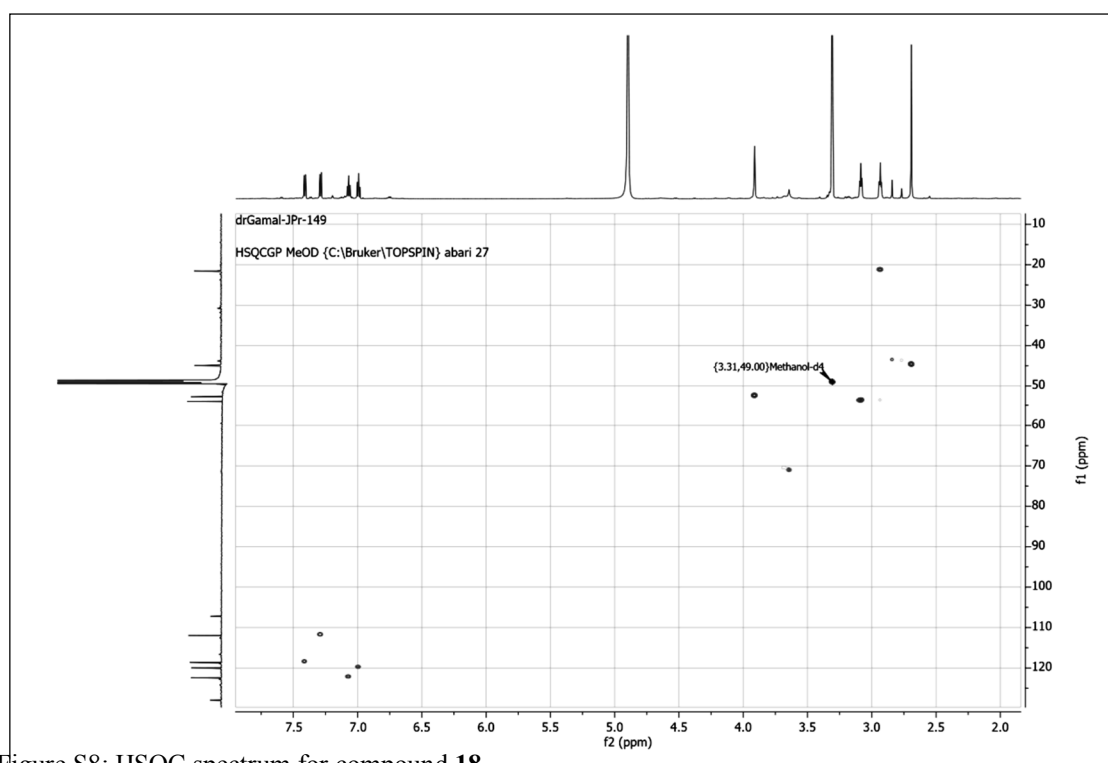


Figure S8: HSQC spectrum for compound **18**

Table S9: Analgesic effect of isolated compounds by using acetic acid-induced writhing in mice

Treatments (n=6)	Dose (mg/kg)	Number of writhing in 20 min	% Inhibition
Control (20% acetic acid)	0.1 ml	36.00±1.15	-
No-2	5	34.33±1.28	4.62
No-2	10	34.50±1.31	4.16
No-6	5	24.66±1.20***	31.48
No-6	10	17.00±0.85***	52.77
No-5	5	18.16±0.47***	49.53
No-5	10	13.33±0.49***	62.96
No-11	5	36.66±0.66	1.85
No-11	10	32.50±0.92*	9.72
No-14	5	30.83±1.13*	14.35
No-14	10	27.50±0.76***	23.61
No-13	5	21.16±0.98***	41.20
No-13	10	15.00±0.57***	58.33
No-16	5	26.83±1.04***	25.46
No-16	10	25.33±0.66***	29.62
No-3	5	35.66±1.70	•
No-3	10	32.33±1.42	10.18
No-4	5	23.16±1.01***	35.64
No-4	10	18.33±0.88***	49.07
No-1	5	24.33±1.30***	32.40
No-1	10	18.16±0.94***	49.53
No-8	5	37.83±1.01	•
No-8	10	37.16±1.32	•
No-9	5	34.00±1.36	5.55
No-9	10	30.00±1.06**	16.16
No-10	5	26.50±0.88***	26.38
No-10	10	24.16±0.94***	32.87
No-21	5	38.00±0.96	•
No-21	10	37.00±0.96	•
No-22	5	24.00±1.48***	33.33
No-22	10	12.33±0.55***	65.74
Indomethacin	4	9.83±0.60***	72.68

All values represent mean ± SEM. *p<0.05, **p<0.01, ***p<0.001; ANOVA, followed by Dunnett's multiple comparison test, compounds and indomethacin groups compared with acetic acid groups

•% inhibition is very low or no effect

Table S10: Analgesic effect of isolated compounds by using hot plate method in mice

Treatments	Dose mg/kg	Reaction time (sec.) pre-drug	Reaction time (sec.) post-drug					
			30 min.	% Change	60 min.	% Change	120 min.	% Change
No-2	5	7.16±0.30	6.83±0.30	4.65	7.66±0.33	6.97	7.66±0.33	6.97
No-2	10	7.00±0.36	7.83±0.30	9.30	7.66±0.33	6.97	8.33±0.42*	16.27
No-6	5	8.00±0.36	8.66±0.33	8.33	10.16±0.30***	27.08	11.66±0.42***	45.83
No-6	10	7.83±0.30	11.00±0.36***	40.25	12.66±0.33***	61.70	13.66±0.42***	74.46
No-5	5	7.83±0.30	9.33±0.49*	19.14	10.50±0.42***	30.04	11.33±0.33***	44.68
No-5	10	7.66±0.49	11.00±0.36***	40.25	12.66±0.33***	61.70	13.00±0.26***	65.95
No-11	5	7.83±0.30	7.33±0.42	•	7.66±0.33	•	8.33±0.33	6.38
No-11	10	7.33±0.33	8.00±0.36	•	7.83±0.30	•	8.83±0.30**	20.45
No-14	5	7.50±0.42	7.83±0.30	4.44	8.16±0.30	8.88	9.16±0.47*	22.22
No-14	10	7.16±0.30	7.83±0.30	9.30	8.33±0.33*	16.27	9.66±0.42***	34.88
No-13	5	8.00±0.36	9.33±0.33*	16.66	10.66±0.42***	33.33	11.00±0.36***	37.50
No-13	10	7.83±0.30	11.00±0.36***	40.25	11.66±0.42***	48.93	12.50±0.61***	59.57
No-16	5	7.16±0.30	8.00±0.36	11.62	9.00±0.36**	25.58	10.16±0.30***	41.86
No-16	10	7.16±0.30	8.66±0.42*	20.93	10.83±0.40***	51.16	11.00±0.36***	53.48
No-3	5	7.00±0.36	7.33±0.33	4.76	7.16±0.30	2.38	8.33±0.42*	19.04
No-3	10	7.00±0.36	7.33±0.33	4.76	7.83±0.30	11.90	9.00±0.36**	28.57
No-4	5	7.16±0.30	7.66±0.33	6.97	8.50±0.42*	18.60	9.83±0.47***	37.20
No-4	10	7.50±0.42	10.00±0.57**	33.33	10.50±0.42***	40.00	13.00±0.36***	73.33
No-1	5	7.66±0.33	7.50±0.42	•	7.66±0.33	•	8.00±0.44	4.34
No-1	10	7.50±0.42	7.66±0.49	2.22	7.83±0.40	4.44	8.33±0.49	11.11
No-8	5	7.00±0.36	7.33±0.33	4.76	7.50±0.42	7.14	8.16±0.40	16.16
No-8	10	7.16±0.30	7.66±0.49	6.97	8.16±0.30*	13.95	9.00±0.36**	25.58
No-9	5	6.83±0.30	7.00±0.36	2.43	7.83±0.30*	14.63	8.16±0.40*	19.51
No-9	10	7.66±0.33	7.83±0.30	2.17	8.66±0.42*	13.04	9.83±0.47**	28.26
No-10	5	7.16±0.30	8.00±0.25	11.62	8.66±0.42*	20.93	10.16±0.30***	41.86
No-10	10	7.00±0.36	9.00±0.25**	28.57	10.50±0.34***	50.00	12.33±0.49***	76.19
No-21	5	6.83±0.30	7.00±0.36	2.43	7.16±0.30	4.87	8.00±0.36	17.07
No-21	10	7.00±0.36	7.66±0.33	8.52	7.50±0.42	7.14	7.83±0.40	11.90
No-22	5	7.00±0.36	7.83±0.30	11.90	8.66±0.42*	23.89	10.33±0.42***	47.61
No-22	10	7.00±0.36	8.50±0.34*	21.42	9.16±0.40**	30.95	12.50±0.42***	78.57
Indomethacin	4	7.16±0.30	10.16±0.30***	41.86	12.83±0.40***	79.06	14.66±0.33***	104.65

All values represent mean ± SEM. *p<0.05, **<0.01, ***p<0.001; ANOVA, followed by Dunnett's multiple comparison test, post-drug compared with pre-drug group

•% inhibition is very low or no effect

Table S11: Analgesic effect of isolated Compounds by using tail flick method in mice

Treatments	Dose mg/kg	Reaction time (s) pre-drug	Reaction time (s) post-drug					
			30 min.	% Change	60 min.	% Change	120 min.	% Change
No-2	5	5.20±0.19	5.11±0.18	•	5.30±0.23	•	5.16±0.12	•
No-2	10	5.10±0.27	5.13±0.12	•	5.28±0.20	3.59	5.08±0.08	•
No-6	5	5.15±0.13	5.45±0.13	5.82	5.88±0.13**	14.23	5.96±0.15**	15.85
No-6	10	5.41±0.16	5.98±0.10*	10.45	6.70±0.10***	23.69	7.60±0.37***	40.30
No-5	5	4.93±0.13	6.06±0.07***	22.97	6.48±0.16***	31.41	7.00±0.09***	41.89
No-5	10	5.28±0.12	6.78±0.11***	28.39	7.33±0.15***	38.80	7.85±0.17***	45.58
No-11	5	5.20±0.15	5.01±0.13	•	5.11±0.10	•	5.25±0.09	•
No-11	10	5.25±0.19	5.11±0.13	•	5.05±0.11	•	5.26±0.11	•
No-14	5	4.93±0.12	4.86±0.08	•	4.98±0.08	•	5.18±0.11	•
No-14	10	5.05±0.13	5.13±0.14	•	5.38±0.11	6.60	5.76±0.18*	14.19
No-13	5	4.96±0.11	5.30±0.10	6.71	6.15±0.11***	23.82	6.53±0.18***	31.54
No-13	10	5.15±0.10	6.01±0.16***	16.82	6.95±0.19***	34.95	7.93±0.14***	54.04
No-16	5	5.21±0.13	5.96±0.12**	14.37	7.26±0.56**	39.55	7.00±0.23***	34.18
No-16	10	5.10±0.11	6.80±0.14***	33.33	7.25±0.13***	42.15	7.83±0.17***	53.59
No-3	5	5.10±0.12	5.13±0.10	*	5.35±0.15	4.90	5.76±0.14**	13.07
No-3	10	5.06±0.14	5.46±0.21	7.89	5.41±0.18	6.90	6.10±0.12***	20.39
No-4	5	5.11±0.09	6.01±0.20**	17.58	6.63±0.17***	29.64	7.18±0.14***	40.39
No-4	10	5.26±0.19	6.70±0.26**	27.21	7.68±0.15***	45.88	8.30±0.23***	57.59
No-1	5	4.83±0.25	6.03±0.13**	28.82	6.31±0.15***	30.68	7.00±0.09***	44.82
No-1	10	5.08±0.12	7.21±0.14***	41.96	7.86±0.17***	54.75	8.50±0.26***	67.21
No-8	5	4.86±0.12	5.08±0.11	4.45	5.36±0.13*	10.27	5.50±0.16*	13.01
No-8	10	5.15±0.19	5.16±0.09	•	5.41±0.12	5.17	5.75±0.09*	11.65
No-9	5	4.98±0.14	5.16±0.14	3.67	5.30±0.10	6.35	5.85±0.12***	17.39
No-9	10	5.51±0.17	5.86±0.11	6.34	6.05±0.12*	9.66	6.11±0.09***	10.87
No-10	5	5.05±0.13	5.88±0.15**	16.50	6.68±0.11***	32.34	7.10±0.08***	40.59
No-10	10	5.10±0.14	6.73 ±0.16***	32.02	7.15±0.25***	40.19	8.18±0.11***	60.45
No-21	5	5.03±0.25	5.18±0.18	2.98	5.26±0.14	4.63	5.15±0.18	9.60
No-21	10	5.13±0.13	5.23±0.12	•	5.96±0.08***	16.23	6.25±0.08***	21.75
No-22	5	5.46±0.19	6.63±0.28**	21.34	7.00±0.25***	28.04	7.56±0.12***	38.10
No-22	10	5.05±0.14	7.06±0.24***	39.93	7.98±0.10***	58.08	8.45±0.16***	67.32
Indomethacin	4	5.31±0.15	7.65±0.24***	43.48	8.05±0.25***	51.41	10.40±0.42***	95.61

All values represent mean ± SEM. *p<0.05, **<0.01, ***p<0.001; ANOVA, followed by Dunnett's multiple comparison test, the post-drug group was compared with the pre-drug group

•% inhibition is very low or no effect

Table S12: Effect of isolated compounds on carrageenan-induced paw edema in albino rats

Treatments	Dose mg/kg	Before carrageenan	3 h after carrageenan	Net	% Inhibition
Only carrageenan	0.2 ml	1.06±0.02	1.66±0.01***	0.60±0.007	-
No-2	5	1.06±0.02	1.67±0.0***	0.60±0.008	•
No-2	10	1.05±0.02	1.64±0.01***	0.59±0.04	•
No-6	5	1.08±0.02	1.48±0.02***	0.39±0.009***	33.97
No-6	10	1.07±0.02	1.36±0.02***	0.29±0.01***	51.10
No-5	5	1.08±0.02	1.48±0.02***	0.32±0.02***	45.85
No-5	10	1.07±0.01	1.36±0.02***	0.29±0.01***	51.38
No-11	5	1.04±0.02	1.65±0.01***	0.60±0.02	•
No-11	10	1.07±0.02	1.67±0.01***	0.60±0.001	•
No-14	5	1.10±0.01	1.66±0.02***	0.56±0.02	6.35
No-14	10	1.09±0.007	1.64±0.01***	0.54±0.02*	10.22
No-13	5	1.06±0.02	1.50±0.02***	0.44±0.02***	26.51
No-13	10	1.02±0.03	1.33±0.03***	0.31±0.009***	48.61
No-16	5	1.06±0.002	1.50±0.002***	0.31±0.003***	47.23
No-16	10	1.02±0.03	1.33±0.03***	0.27±0.007***	55.24
No-3	5	1.09±0.008	1.59±0.01***	0.56±0.01*	7.18
No-3	10	1.04±0.03	1.56±0.02***	0.52±0.02**	13.53
No-4	5	1.07±0.02	1.60±0.02***	0.52±0.01**	13.53
No-4	10	1.08±0.01	1.41±0.01***	0.30±0.004***	49.17
No-1	5	1.05±0.03	1.54±0.02***	0.49±0.04*	17.95
No-1	10	1.09±0.01	1.36±0.01***	0.27±0.009***	54.94
No-8	5	1.09±0.009	1.68±0.001***	0.59±0.01	2.20
No-8	10	1.13±0.01	1.65±0.02***	0.52±0.03*	12.98
No-9	5	1.04±0.02	1.61±0.01***	0.57±0.01	5.52
No-9	10	1.08±0.02	1.62±0.01***	0.54±0.01**	10.22
No-10	5	1.00±0.03	1.48±0.01***	0.48±0.03**	20.44
No-10	10	1.10±0.006	1.31±0.01***	0.21±0.01***	64.91
No-21	5	1.09±0.03	1.66±0.009***	0.56±0.01*	6.35
No-21	10	1.06±0.02	1.62±0.02***	0.55±0.01*	8.01
No-22	5	1.10±0.007	1.43±0.02***	0.33±0.007***	44.47
No-22	10	1.08±0.01	1.38±0.009***	0.30±0.008***	50.27
Phenylbutazone	100	1.03±0.02	1.22±0.02***	0.18±0.007***	69.06

All values represent mean ± SEM. *p<0.05, **p<0.01, ***p<0.001; ANOVA, followed by Dunnett's multiple comparison test. The compound and phenylbutazone groups were compared with the carrageenan groups.

•% inhibition is very low or no effect

Table S13: Effect of isolated compounds on yeast-induced hyperthermia in mice

Treatments	Dose mg/kg	Normal rector temperature	Rectal temperature after yeast administration 20 ml/kg of 20%	Rectal temperature after drug administration 20 ml/kg of 20%		
				30 min	60 min	120 min
No-2	5	35.06±0.12	38.83±0.10***	38.80±0.12	38.73±0.14	38.20±0.12*
No-2	10	35.16±0.12	38.65±0.12***	38.51±0.15	38.45±0.13	38.08±0.12*
No-6	5	35.20±0.11	38.76±0.14***	38.25±0.08*	38.26±0.12*	37.78±0.11***
No-6	10	35.16±0.10	38.81±0.12***	37.91±0.11***	37.56±0.17***	36.98±0.15***
No-5	5	35.53±0.11	38.88±0.10***	38.33±0.05***	37.93±0.07***	37.81±0.16***
No-5	10	35.33±0.11	38.78±0.19***	37.91±0.16**	37.40±0.17***	37.00±0.09***
No-11	5	35.38±0.08	38.85±0.16***	38.71±0.18	38.68±0.11	38.56±0.16*
No-11	10	35.28±0.08	38.70±0.11***	38.60±0.12	38.53±0.13	38.25±0.09*
No-14	5	35.40±0.16	38.81±0.12***	38.68±0.15	38.61±0.15	38.43±0.13*
No-14	10	35.93±0.58	38.96±0.12***	38.81±0.11	38.56±0.11	38.43±0.12*
No-13	5	35.53±0.10	38.70±0.16***	38.41±0.15	37.96±0.12**	37.36±0.12***
No-13	10	35.56±0.12	38.85±0.11***	37.90±0.09***	37.28±0.10***	37.03±0.09***
No-16	5	35.35±0.11	38.75±0.21***	38.21±0.07*	37.90±0.09**	37.58±0.016**
No-16	10	35.38±0.18	38.80±0.20***	37.83±0.12**	37.11±0.09***	36.83±0.10***
No-3	5	35.65±0.11	38.93±0.13***	38.93±0.15	38.80±0.13	38.66±0.11*
No-3	10	35.71±0.11	38.88±0.13***	38.73±0.13	38.51±0.07*	38.60±0.14*
No-4	5	35.83±0.12	38.90±0.08***	38.91±0.12	38.60±0.11	38.48±0.14*
No-4	10	35.76±0.13	38.91±0.07***	38.88±0.14	38.36±0.11**	38.31±0.11**
No-1	5	35.66±0.08	38.88±0.13***	38.28±0.09**	37.98±0.15**	37.43±0.15***
No-1	10	35.55±0.16	38.88±0.11***	38.13±0.13**	37.58±0.17***	37.06±0.17***
No-8	5	35.56±0.23	38.88±0.11***	38.80±0.13	38.78±0.13	38.65±0.11*
No-8	10	35.61±0.18	38.75±0.15***	38.53±0.14	38.61±0.11	38.33±0.18*
No-9	5	35.66±0.12	38.76±0.16***	38.75±0.11	38.61±0.13	38.30±0.10*
No-9	10	35.60±0.12	38.81±0.12***	38.76±0.11	38.63±0.13	38.00±0.09***
No-10	5	35.83±0.09	38.86±0.14***	38.31±0.09*	37.81±0.13***	37.26±0.09***
No-10	10	35.78±0.12	38.85±0.15***	37.83±0.11***	37.40±0.17***	37.13±0.18***
No-21	5	35.63±0.16	38.85±0.13***	38.85±0.12	38.71±0.11	38.53±0.12*
No-21	10	35.68±0.17	38.86±0.17***	38.66±0.12	38.45±0.14	38.20±0.12*
No-22	5	35.70±0.11	38.81±0.11***	38.10±0.10***	37.80±0.15***	37.20±0.11***
No-22	10	35.75±0.09	38.86±0.12***	38.23±0.10**	37.11±0.10***	36.73±0.13***
Indomethacin	4	35.71±0.18	38.95±0.10***	37.10±0.09***	36.85±0.18***	36.33±0.11***

All values represent mean ± SEM. *p<0.05, **<0.01, ***p<0.001; ANOVA, followed by Dunnett's multiple comparison test. The pre-drug group was compared with the normal group, and the post-drug group was compared with the yeast administration groups.

Table S14: % Inhibition of nitric oxide scavenging activity for isolated compounds at different concentrations

Treatments	(% Inhibition $\pm$ SD)				
	Concentration				
	20 μ g/ml	40 μ g/ml	60 μ g/ml	80 μ g/ml	100 μ g/ml
Compound-2	30.15 $\pm$ 14.36	54.43 $\pm$ 3.14	63.96 $\pm$ 10.70	71.60 $\pm$ 10.48	76.83 $\pm$ 5.01
Compound-6	10.56 $\pm$ 5.34	23.09 $\pm$ 3.04	33.50 $\pm$ 8.52	50.03 $\pm$ 4.00	56.54 $\pm$ 6.03
Compound-5	5.16 $\pm$ 2.48	10.43 $\pm$ 3.25	19.36 $\pm$ 8.72	21.63 $\pm$ 10.28	27.00 $\pm$ 7.85
Compound-11	7.40 $\pm$ 4.56	20.06 $\pm$ 8.58	36.71 $\pm$ 16.53	50.50 $\pm$ 10.21	57.00 $\pm$ 10.21
Compound-14	17.86 $\pm$ 15.85	28.10 $\pm$ 22.28	42.40 $\pm$ 23.65	59.16 $\pm$ 14.84	70.36 $\pm$ 14.73
Compound-13	10.40 $\pm$ 6.32	14.16 $\pm$ 4.35	25.61 $\pm$ 5.18	30.04 $\pm$ 3.35	33.06 $\pm$ 1.86
Compound-16	6.66 $\pm$ 2.10	11.26 $\pm$ 0.28	19.20 $\pm$ 6.40	21.86 $\pm$ 5.35	25.61 $\pm$ 5.18
Compound-3	7.40 $\pm$ 4.56	12.63 $\pm$ 5.43	19.43 $\pm$ 6.21	28.46 $\pm$ 12.00	38.30 $\pm$ 5.63
Compound-4	23.86 $\pm$ 8.91	41.43 $\pm$ 7.77	48.16 $\pm$ 7.16	66.03 $\pm$ 8.24	77.36 $\pm$ 4.22
Compound-1	11.46 $\pm$ 5.00	27.90 $\pm$ 0.45	44.30 $\pm$ 6.76	61.53 $\pm$ 2.56	71.66 $\pm$ 0.70
Compound-8	7.50 $\pm$ 1.55	15.75 $\pm$ 0.44	42.61 $\pm$ 10.72	50.17 $\pm$ 9.22	63.67 $\pm$ 12.85
Compound-9	15.06 $\pm$ 7.50	29.30 $\pm$ 2.98	38.27 $\pm$ 2.98	50.43 $\pm$ 7.23	63.67 $\pm$ 12.85
Compound-10	33.31 $\pm$ 19.72	45.38 $\pm$ 20.11	56.55 $\pm$ 9.42	64.73 $\pm$ 4.35	75.26 $\pm$ 5.54
Compound-21	39.97 $\pm$ 7.66	54.97 $\pm$ 6.68	61.70 $\pm$ 2.95	63.18 $\pm$ 1.66	67.76 $\pm$ 5.75
Compound-22	23.86 $\pm$ 8.91	41.43 $\pm$ 7.77	48.16 $\pm$ 7.16	66.03 $\pm$ 8.24	77.60 $\pm$ 4.22
Ascorbic Acid	64.38 $\pm$ 15.47	66.64 $\pm$ 15.42	78.13 $\pm$ 3.22	85.68 $\pm$ 1.69	87.23 $\pm$ 0.98

The statistical significant was calculated from one-way ANOVA, and all parameters are expressed as mean $\pm$ SD of three independent measurements.