

# ***Molecules***

## **In Vivo and In Vitro Anti-Arthritic Effects of Cardenolide-Rich and Caffeoylquinic Acid-Rich Fractions of *Periploca Forrestii***

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**Table 1-a Changes of the weight of rats from day0 to 11 ( $\bar{x} \pm s$ ,  $n = 8$ )**

Groups	day 0	day 1	day 3	day 5	day 7	day 9	day 11
non-treated control	138.16±8.71	163.02±9.64	164.05±9.64	165.50±9.32	184.46±10.16	175.20±9.50	182.38±6.66
CIA-treated control	135.91±8.09	159.90±12.93	159.9±12.93	167.30±7.52	184.97±12.40	172.65±14.37	174.16±9.80 <sup>#</sup>
TGT (37.5 mg/kg)	137.22±9.15	165.40±12.07	165.40±12.07	163.49±12.18	184.49±15.31	174.67±13.55	178.38±9.30
CQAF (125 mg/kg)	134.17±7.37	159.90±13.60	160.90±13.20	164.50±12.90	187.60±15.30	170.85±13.26	176.05±8.34
CQAF (250 mg/kg)	135.71±6.53	157.50±10.80	161.00±9.80	161.90±10.10	182.60±10.90	169.92±8.75	177.73±4.93
CQAF (500 mg/kg)	136.97±8.27	156.00±18.70	159.30±19.60	165.50±19.10	179.80±24.40	173.37±9.76	179.52±6.48
CDLF (125 mg/kg)	135.17±8.37	161.21±8.22	161.21±8.22	167.49±9.11	185.73±9.26	169.85±16.26	174.05±8.44
CDLF (250 mg/kg)	134.71±6.93	157.43±6.06	157.43±6.06	162.29±6.68	181.67±8.44	169.72±8.85	174.73±3.93
CDLF (500 mg/kg)	135.97±8.07	156.90±11.51	156.90±11.51	163.98±11.30	183.84±14.72	173.37±8.76	180.52±4.48

**Table 1-b Changes of the weight of rats from day0 to 11 ( $\bar{x} \pm s$ ,  $n = 8$ )**

Groups	Groups (mg/kg)	day 15	day 19	day 21	day 25	day 28
non-treated control	non-treated control	205.53±8.09	235.25±9.08	240.62±10.88	251.89±12.07	263.63±10.63
CIA-treated control	CIA-treated control	196.47±11.05 <sup>#</sup>	217.43±9.99 <sup>##</sup>	216.21±14.26 <sup>##</sup>	220.82±21.53 <sup>##</sup>	211.04±35.17 <sup>##</sup>
TGT (37.5 mg/kg)	TGT (37.5)	201.49±13.90	231.64±14.98 <sup>*</sup>	230.11±17.42	242.66±18.84 <sup>*</sup>	247.29±21.10 <sup>*</sup>
CQAF (125 mg/kg)	CQAF (125)	197.23±12.88	220.48±18.43	215.87±10.24	228.54±12.58	230.10±16.12 <sup>*</sup>
CQAF (250 mg/kg)	CQAF (250)	200.90±6.39	226.47±7.47	229.76±7.69	235.68±15.72 <sup>*</sup>	250.20±6.67 <sup>**</sup>
CQAF (500 mg/kg)	CQAF (500)	203.57±5.84	228.85±9.82 <sup>*</sup>	233.28±12.59 <sup>*</sup>	243.61±15.61 <sup>**</sup>	258.26±16.02 <sup>**</sup>
CDLF (125 mg/kg)	CDLF (125)	193.23±13.88	222.48±19.43	216.47±13.24	230.24±22.58	233.10±18.22 <sup>*</sup>
CDLF (250 mg/kg)	CDLF (250)	199.90±5.39	228.57±8.47 <sup>*</sup>	230.79±7.69 <sup>*</sup>	239.99±20.68 <sup>*</sup>	255.20±6.97 <sup>**</sup>
CDLF (500 mg/kg)	CDLF (500)	204.27±5.94	231.95±12.82 <sup>*</sup>	237.27±15.89 <sup>**</sup>	248.91±15.21 <sup>**</sup>	261.29±26.02 <sup>**</sup>

Values are represented as the mean  $\pm$  SD ( $n=8$ ). <sup>#</sup> $P<0.05$ , <sup>##</sup>  $P<0.01$  when compared with the non-treated control group; <sup>\*</sup> $P<0.05$ , <sup>\*\*</sup> $P<0.01$  when compared with the CIA-treated control group.

**Table 2. Effects of CDLF and CQAF on hind paw edema in arthritic rats induced by collagen**

Groups (mg/kg)	The changes of hind paw edema (mL)											
	day 0	day 1	day 3	day 5	day 7	day 9	day 11	day 15	day 19	day 21	day 25	day 28
non-treated control	1.53±0.09	1.54±0.07	1.50±0.11	1.55±0.09	1.61±0.09	1.54±0.09	1.55±0.05	1.54±0.08	1.56±0.08	1.58±0.07	1.55±0.03	1.57±0.04
CIA-treated control	1.54±0.08	2.59±0.21 <sup>##</sup>	2.25±0.15 <sup>##</sup>	2.16±0.19 <sup>##</sup>	2.06±0.09 <sup>##</sup>	3.45±0.43 <sup>##</sup>	3.66±0.33 <sup>##</sup>	3.88±0.23 <sup>##</sup>	3.91±0.29 <sup>##</sup>	3.87±0.18 <sup>##</sup>	4.10±0.15 <sup>##</sup>	4.08±0.12 <sup>##</sup>
TGT (37.5)	1.59±0.11	2.55±0.27	2.31±0.24	2.17±0.22	2.12±0.17	3.76±0.67	3.39±0.58	3.09±0.57 <sup>*</sup>	2.97±0.44 <sup>**</sup>	2.97±0.51 <sup>**</sup>	3.13±0.91 <sup>**</sup>	2.93±0.48 <sup>**</sup>
CQAF (125)	1.57±0.06	2.46±0.26	2.32±0.40	2.06±0.30	2.11±0.36	3.18±0.66	3.49±0.30	3.56±0.24	3.64±0.21	3.74±0.24	3.99±0.13	3.92±0.13 <sup>*</sup>
CQAF (250)	1.49±0.09	2.47±0.24	2.29±0.29	2.14±0.23	2.10±0.24	2.96±0.45	3.12±0.39	2.73±0.30 <sup>*</sup>	2.64±0.28 <sup>**</sup>	2.58±0.28 <sup>**</sup>	2.44±0.15 <sup>**</sup>	2.80±0.33 <sup>**</sup>
CQAF (500)	1.53±0.10	2.55±0.12	2.35±0.07	2.24±0.18	2.11±0.16	2.88±0.67	2.92±0.56 <sup>*</sup>	2.69±0.52 <sup>*</sup>	2.71±0.57 <sup>**</sup>	2.73±0.35 <sup>**</sup>	2.52±0.49 <sup>**</sup>	2.52±0.39 <sup>**</sup>
CDLF (125)	1.51±0.10	2.50±0.16	2.46±0.28	2.20±0.28	2.24±0.21	3.61±0.51	3.40±0.40	3.67±0.36	3.68±0.26	3.90±0.18	3.87±0.21	3.88±0.20 <sup>*</sup>
CDLF (250)	1.51±0.08	2.54±0.16	2.26±0.18	2.09±0.20	2.02±0.15	3.30±0.27	3.25±0.34	3.05±0.23	2.99±0.34 <sup>**</sup>	2.74±0.27 <sup>**</sup>	2.75±0.35 <sup>**</sup>	2.81±0.22 <sup>**</sup>
CDLF (500)	1.53±0.05	2.39±0.29	2.16±0.18	1.98±0.20	2.02±0.18	3.00±0.28	3.19±0.24	2.94±0.17 <sup>*</sup>	2.81±0.26 <sup>**</sup>	2.70±0.17 <sup>**</sup>	2.75±0.20 <sup>**</sup>	2.67±0.12 <sup>**</sup>

Values are represented as the mean ± SD (*n*=8). <sup>##</sup>*P*<0.01 when compared with the non-treated control group; <sup>\*</sup>*P*<0.05, <sup>\*\*</sup>*P*<0.01 when compared with the CIA-treated control group.

**Table 3. Effects of CDLF and CQAF on serum RF, TNF- $\alpha$ , IL-6, IL-1 $\beta$ , and PGE<sub>2</sub> in the CIA rats ( $n = 8$ )**

Group		RF (ng/L)	TNF- $\alpha$ (ng/L)	IL-6 (ng/L)	IL-1 $\beta$ (ng/L)	PGE <sub>2</sub> (ng/L)
Non-treated control	-	158.405 $\pm$ 39.553	90.953 $\pm$ 6.256	14.962 $\pm$ 2.726	5.298 $\pm$ 0.719	184.489 $\pm$ 40.919
CIA-treated control	-	320.242 $\pm$ 38.792 <sup>##</sup>	124.253 $\pm$ 16.238 <sup>##</sup>	19.619 $\pm$ 1.224 <sup>##</sup>	6.700 $\pm$ 0.668 <sup>##</sup>	243.784 $\pm$ 10.426 <sup>##</sup>
TGT	37.5 mg/kg	211.564 $\pm$ 36.506 <sup>**</sup>	91.614 $\pm$ 7.383 <sup>**</sup>	15.425 $\pm$ 2.278 <sup>**</sup>	5.199 $\pm$ 0.718 <sup>**</sup>	186.212 $\pm$ 28.079 <sup>**</sup>
	125 mg/kg	292.285 $\pm$ 31.388 <sup>*</sup>	102.201 $\pm$ 11.269 <sup>**</sup>	17.029 $\pm$ 1.934 <sup>**</sup>	5.894 $\pm$ 0.696 <sup>**</sup>	220.118 $\pm$ 18.604 <sup>**</sup>
CQAF	250 mg/kg	211.090 $\pm$ 29.984 <sup>**</sup>	96.121 $\pm$ 9.893 <sup>**</sup>	16.443 $\pm$ 1.808 <sup>**</sup>	5.712 $\pm$ 0.597 <sup>**</sup>	207.881 $\pm$ 32.322 <sup>**</sup>
	500 mg/kg	177.832 $\pm$ 20.065 <sup>**</sup>	90.916 $\pm$ 12.997 <sup>**</sup>	15.807 $\pm$ 1.199 <sup>**</sup>	5.401 $\pm$ 0.643 <sup>**</sup>	195.132 $\pm$ 31.437 <sup>**</sup>
CDLF	125 mg/kg	298.744 $\pm$ 54.909 <sup>*</sup>	107.901 $\pm$ 9.421 <sup>**</sup>	17.629 $\pm$ 1.513 <sup>**</sup>	6.214 $\pm$ 0.545 <sup>**</sup>	233.718 $\pm$ 11.111 <sup>*</sup>
	250 mg/kg	257.517 $\pm$ 45.910 <sup>**</sup>	103.021 $\pm$ 7.383 <sup>**</sup>	17.043 $\pm$ 0.903 <sup>**</sup>	6.012 $\pm$ 0.492 <sup>**</sup>	216.581 $\pm$ 22.107 <sup>**</sup>
	500 mg/kg	175.539 $\pm$ 52.484 <sup>**</sup>	95.916 $\pm$ 7.128 <sup>**</sup>	16.307 $\pm$ 1.221 <sup>**</sup>	5.601 $\pm$ 0.719 <sup>**</sup>	208.332 $\pm$ 10.164 <sup>**</sup>

The data are presented as the mean  $\pm$  SD. <sup>##</sup> $P < 0.01$  when compared with the non-treated control group;

<sup>\*</sup> $P < 0.05$ , <sup>\*\*</sup> $P < 0.01$  when compared with the CIA-treated control group.

**Table 4. Effects of CDLF and CQAF on serum NO, MDA, and SOD in CIA rats ( $n = 8$ )**

Group		NO ( $\mu$ mol/L)	MDA ( $\mu$ mol/L)	SOD (%)
Non-treated control	-	6.479 $\pm$ 0.731	5.227 $\pm$ 0.413	61.658 $\pm$ 6.626
CIA-treated control	-	10.350 $\pm$ 1.219 <sup>##</sup>	6.805 $\pm$ 0.390 <sup>##</sup>	45.092 $\pm$ 7.565 <sup>##</sup>
TGT	37.5 mg/kg	6.938 $\pm$ 1.757 <sup>**</sup>	5.293 $\pm$ 0.394 <sup>**</sup>	52.179 $\pm$ 8.663 <sup>**</sup>
	125 mg/kg	7.553 $\pm$ 1.660 <sup>**</sup>	6.214 $\pm$ 0.384	48.534 $\pm$ 7.314
CQAF	250 mg/kg	7.176 $\pm$ 1.052 <sup>**</sup>	5.895 $\pm$ 0.407 <sup>*</sup>	54.448 $\pm$ 9.716 <sup>**</sup>
	500 mg/kg	6.396 $\pm$ 1.392 <sup>**</sup>	5.522 $\pm$ 0.260 <sup>**</sup>	63.097 $\pm$ 7.948 <sup>**</sup>
CDLF	125 mg/kg	8.153 $\pm$ 1.369 <sup>**</sup>	6.195 $\pm$ 0.467	45.784 $\pm$ 4.787
	250 mg/kg	7.476 $\pm$ 0.875 <sup>**</sup>	5.814 $\pm$ 0.395 <sup>*</sup>	52.782 $\pm$ 7.949 <sup>**</sup>
	500 mg/kg	6.896 $\pm$ 1.223 <sup>**</sup>	5.615 $\pm$ 0.522 <sup>*</sup>	62.263 $\pm$ 7.666 <sup>**</sup>

The data are presented as the mean  $\pm$  SD. <sup>##</sup> $P < 0.01$  when compared with the non-treated control group;

<sup>\*</sup> $P < 0.05$ , <sup>\*\*</sup> $P < 0.01$  when compared with the CIA-treated control group.

**Table 5. Effects of CQAF on the mRNA expressions of IL-1 $\beta$ , IL-6, iNOS, and COX-2 in MH7A cells**

Group		Relative mRNA expression			
		IL-6	IL-1 $\beta$	iNOS	COX-2
Non-treated control	-	0.006 $\pm$ 0.002	0.078 $\pm$ 0.005	0.016 $\pm$ 0.001	0.167 $\pm$ 0.008
TNF- $\alpha$	50 ng/mL	1.096 $\pm$ 0.190 <sup>##</sup>	1.359 $\pm$ 0.113 <sup>##</sup>	1.096 $\pm$ 0.175 <sup>##</sup>	1.631 $\pm$ 0.127 <sup>##</sup>
	100 $\mu$ g/mL	0.427 $\pm$ 0.038 <sup>**</sup>	0.970 $\pm$ 0.157 <sup>**</sup>	0.363 $\pm$ 0.079 <sup>**</sup>	0.870 $\pm$ 0.046 <sup>**</sup>
CQAF	200 $\mu$ g/mL	0.034 $\pm$ 0.003 <sup>**</sup>	0.330 $\pm$ 0.078 <sup>**</sup>	0.216 $\pm$ 0.014 <sup>**</sup>	0.706 $\pm$ 0.099 <sup>**</sup>
	400 $\mu$ g/mL	0.011 $\pm$ 0.001 <sup>**</sup>	0.204 $\pm$ 0.017 <sup>**</sup>	0.116 $\pm$ 0.034 <sup>**</sup>	0.653 $\pm$ 0.016 <sup>**</sup>

The data are presented as the mean  $\pm$  SD ( $n = 3$ ). <sup>##</sup> $P < 0.01$  when compared with the non-treated control group; <sup>\*\*</sup> $P < 0.01$  when compared with the TNF- $\alpha$  group.

**Table 6. Effects of CDLF on the mRNA expressions of IL-1 $\beta$ , IL-6, iNOS, and COX-2 in MH7A cells**

Group		Relative mRNA expressions			
		IL-6	IL-1 $\beta$	iNOS	COX-2
Non-treated control	-	0.007 $\pm$ 0.002	0.279 $\pm$ 0.034	0.015 $\pm$ 0.003	0.023 $\pm$ 0.031
TNF- $\alpha$	50 ng/mL	1.000 $\pm$ 0.069 <sup>#</sup>	1.532 $\pm$ 0.065 <sup>#</sup>	1.000 $\pm$ 0.043 <sup>#</sup>	1.631 $\pm$ 0.114 <sup>#</sup>
	100 $\mu$ g/mL	0.531 $\pm$ 0.085 <sup>**</sup>	1.000 $\pm$ 0.016 <sup>**</sup>	0.735 $\pm$ 0.036 <sup>**</sup>	0.591 $\pm$ 0.117 <sup>**</sup>
CDLF	200 $\mu$ g/mL	0.034 $\pm$ 0.015 <sup>**</sup>	0.806 $\pm$ 0.149 <sup>**</sup>	0.633 $\pm$ 0.028 <sup>**</sup>	0.585 $\pm$ 0.029 <sup>**</sup>
	400 $\mu$ g/mL	0.019 $\pm$ 0.003 <sup>**</sup>	0.679 $\pm$ 0.082 <sup>**</sup>	0.112 $\pm$ 0.005 <sup>**</sup>	0.221 $\pm$ 0.054 <sup>**</sup>

The data are presented as the mean  $\pm$  SD ( $n = 3$ ). <sup>#</sup> $P < 0.01$  when compared with the non-treated control group; <sup>\*\*</sup> $P < 0.01$  when compared with the TNF- $\alpha$  group.

**Table 7. Effects of CQAF on protein expressions of iNOS and COX-2 in MH7A cells ( $n = 3$ )**

Group		Relative density	
		iNOS	COX-2
Non-treated control	-	0.392 $\pm$ 0.018	0.249 $\pm$ 0.031
TNF- $\alpha$	50 ng/mL	0.527 $\pm$ 0.005 <sup>#</sup>	0.412 $\pm$ 0.022 <sup>#</sup>
	100 $\mu$ g/mL	0.271 $\pm$ 0.029 <sup>**</sup>	0.294 $\pm$ 0.020 <sup>**</sup>
CQAF	200 $\mu$ g/mL	0.167 $\pm$ 0.010 <sup>**</sup>	0.189 $\pm$ 0.009 <sup>**</sup>
	400 $\mu$ g/mL	0.143 $\pm$ 0.009 <sup>**</sup>	0.143 $\pm$ 0.009 <sup>**</sup>

The data are presented as the mean  $\pm$  SD. <sup>#</sup> $P < 0.01$  when compared with the non-treated control group; <sup>\*\*</sup> $P < 0.01$  when compared with the TNF- $\alpha$  group.

**Table 8. Effects of CDLF on protein expressions of iNOS and COX-2 in MH7A cells ( $n = 3$ )**

Group		Relative density	
		iNOS	COX-2
Non-treated control	-	0.380 $\pm$ 0.019	0.411 $\pm$ 0.017
TNF- $\alpha$	50 ng/mL	0.508 $\pm$ 0.016 <sup>#</sup>	0.596 $\pm$ 0.021 <sup>#</sup>
	100 $\mu$ g/mL	0.425 $\pm$ 0.019 <sup>**</sup>	0.445 $\pm$ 0.003 <sup>*</sup>
CDLF	200 $\mu$ g/mL	0.428 $\pm$ 0.002 <sup>**</sup>	0.424 $\pm$ 0.005 <sup>*</sup>
	400 $\mu$ g/mL	0.411 $\pm$ 0.032 <sup>**</sup>	0.441 $\pm$ 0.034 <sup>*</sup>

The data are presented as the mean  $\pm$  SD. <sup>#</sup> $P < 0.01$  when compared with the non-treated control group; <sup>\*</sup> $P < 0.05$ , <sup>\*\*</sup> $P < 0.01$  when compared with the TNF- $\alpha$  group.

**Table 9. Effects of CAQF on the expression of the p65 protein and I $\kappa$ B phosphorylation ( $n = 3$ )**

Group		Relative density	
		p-I $\kappa$ B $\alpha$ /I $\kappa$ B $\alpha$	P65
Non-treated control	-	1.109 $\pm$ 0.029	1.387 $\pm$ 0.015
TNF- $\alpha$	50 ng/mL	2.175 $\pm$ 0.096 <sup>#</sup>	2.050 $\pm$ 0.017 <sup>#</sup>
	100 $\mu$ g/mL	1.808 $\pm$ 0.185 <sup>**</sup>	1.628 $\pm$ 0.095 <sup>*</sup>
CAQF	200 $\mu$ g/mL	1.518 $\pm$ 0.026 <sup>**</sup>	1.296 $\pm$ 0.006 <sup>*</sup>
	400 $\mu$ g/mL	0.811 $\pm$ 0.041 <sup>**</sup>	0.752 $\pm$ 0.061 <sup>*</sup>

The data are presented as the mean  $\pm$  SD ( $n = 3$ ). <sup>#</sup> $P < 0.01$  when compared with the non-treated control group; <sup>\*</sup> $P < 0.05$ , <sup>\*\*</sup> $P < 0.01$  when compared with the TNF- $\alpha$  group.

**Table 10. Effects of CDLF on the expression of the p65 protein and I $\kappa$ B phosphorylation ( $n = 3$ )**

Group		Relative density	
		p-I $\kappa$ B $\alpha$ /I $\kappa$ B $\alpha$	P65
Non-treated control	-	0.855 $\pm$ 0.037	0.916 $\pm$ 0.022
TNF- $\alpha$	50 ng/mL	2.346 $\pm$ 0.154 <sup>##</sup>	1.864 $\pm$ 0.019 <sup>##</sup>
	100 $\mu$ g/mL	1.085 $\pm$ 0.039 <sup>**</sup>	1.799 $\pm$ 0.025
CDLF	200 $\mu$ g/mL	1.293 $\pm$ 0.126 <sup>**</sup>	1.847 $\pm$ 0.012
	400 $\mu$ g/mL	0.828 $\pm$ 0.036 <sup>**</sup>	1.783 $\pm$ 0.016

The data are presented as the mean  $\pm$  SD ( $n = 3$ ). <sup>##</sup> $P < 0.01$  when compared with the non-treated control group; <sup>\*\*</sup> $P < 0.01$  when compared with the TNF- $\alpha$  group.

**Table 11. Effects of CQAF on the protein expressions of p38, ERK, and JNK phosphorylation in MH7A cells ( $n = 3$ )**

Group		p-p38/p38 (relative density)	p-JNK/JNK (relative density)	p-ERK/ERK (relative density)
Non-treated control	-	0.551 $\pm$ 0.049	0.996 $\pm$ 0.024	0.905 $\pm$ 0.017
TNF- $\alpha$	50 ng/mL	0.847 $\pm$ 0.043 <sup>##</sup>	1.777 $\pm$ 0.073 <sup>##</sup>	1.436 $\pm$ 0.030 <sup>##</sup>
	100 $\mu$ g/mL	0.819 $\pm$ 0.077	1.277 $\pm$ 0.051 <sup>**</sup>	1.183 $\pm$ 0.065 <sup>**</sup>
CAQF	200 $\mu$ g/mL	0.708 $\pm$ 0.030 <sup>*</sup>	1.015 $\pm$ 0.051 <sup>**</sup>	0.654 $\pm$ 0.018 <sup>**</sup>
	400 $\mu$ g/mL	0.631 $\pm$ 0.015 <sup>**</sup>	0.736 $\pm$ 0.012 <sup>**</sup>	0.560 $\pm$ 0.022 <sup>**</sup>

The data are presented as the mean  $\pm$  SD ( $n = 3$ ). <sup>##</sup> $P < 0.01$  when compared with the non-treated control group; <sup>\*</sup> $P < 0.05$ , <sup>\*\*</sup> $P < 0.01$  when compared with the TNF- $\alpha$  group.

**Table 12. Effects of CDLF on the protein expressions of p38, ERK, and JNK phosphorylation in MH7A cells ( $n = 3$ )**

Group		p-p38/p38 (relative density)	p-JNK/JNK (relative density)	p-ERK/ERK (relative density)
Non-treated control	-	1.628 $\pm$ 0.183	0.746 $\pm$ 0.040	0.296 $\pm$ 0.012
TNF- $\alpha$	50 ng/mL	2.775 $\pm$ 0.040 <sup>##</sup>	1.071 $\pm$ 0.017 <sup>##</sup>	0.704 $\pm$ 0.011 <sup>##</sup>
	100 $\mu$ g/mL	2.155 $\pm$ 0.044 <sup>**</sup>	0.854 $\pm$ 0.021 <sup>**</sup>	0.551 $\pm$ 0.048 <sup>**</sup>
CDLF	200 $\mu$ g/mL	2.134 $\pm$ 0.117 <sup>*</sup>	0.901 $\pm$ 0.025 <sup>**</sup>	0.662 $\pm$ 0.025
	400 $\mu$ g/mL	1.178 $\pm$ 0.014 <sup>**</sup>	0.848 $\pm$ 0.063 <sup>**</sup>	0.477 $\pm$ 0.039 <sup>**</sup>

The data are presented as the mean  $\pm$  SD ( $n = 3$ ). <sup>##</sup> $P < 0.01$  when compared with Non-treated control group; <sup>\*</sup> $P < 0.05$ , <sup>\*\*</sup> $P < 0.01$  when compared with the TNF- $\alpha$  group.

Table 13. Chemical composition of CDLF

chemical formula	RT (min)	m/z (ESI-)	mass error (ppm)	response (ESI-)	adduct	m/z (ESI+)	mass error (ppm)	response (ESI+)	adduct
C <sub>40</sub> H <sub>66</sub> O <sub>17</sub>	9.18	863.4311	3.3	626912	+HCOO	841.4202	1.1	116006	+Na
C <sub>36</sub> H <sub>56</sub> O <sub>13</sub>	9.29	741.3707	0.6	159633	+HCOO, +Cl	719.3608	-0.7	12296	+Na, +K
C <sub>23</sub> H <sub>34</sub> O <sub>6</sub>	9.43	451.2333	-0.9	201326	+HCOO, -H	ND	ND	ND	ND
C <sub>23</sub> H <sub>32</sub> O <sub>5</sub>	9.63	433.223	-0.5	76661	+HCOO, -H, +Cl	389.2316	-1.6	125074	+H, +Na, +K
C <sub>40</sub> H <sub>66</sub> O <sub>16</sub>	9.67	847.4353	2.4	346887	+HCOO	ND	ND	ND	ND
C <sub>23</sub> H <sub>34</sub> O <sub>5</sub>	9.68	435.2386	-0.5	850781	+HCOO, -H, +Cl	391.2471	-2	397796	+H, +Na, +K
C <sub>18</sub> H <sub>34</sub> O <sub>5</sub>	10.04	329.2331	-0.8	544269	-H	ND	ND	ND	ND
C <sub>27</sub> H <sub>44</sub> O <sub>6</sub>	10.43	509.3119	-0.2	128993	+HCOO	487.3029	-0.2	19972	+Na, +K
C <sub>34</sub> H <sub>52</sub> O <sub>9</sub>	11.03	649.3606	2	354449	+HCOO, +Cl	627.3508	0.7	91194	+Na, +K

**Table 14. Chemical composition of CQAF**

Compound	chemical formula	RT (min)	m/z (ESI-)	mass error (ppm)	response (ESI-)	adduct	m/z (ESI+)	mass error (ppm)	response (ESI+)	adduct
Caffeoylquinic acid /isomer	C <sub>16</sub> H <sub>18</sub> O <sub>9</sub>	3.64	353.0875	-0.8	105705	-H	355.1014	-2.6	5575	+H,+Na
Caffeoylquinic acid /isomer	C <sub>16</sub> H <sub>18</sub> O <sub>9</sub>	4.41	353.0876	-0.7	283059	-H	355.1017	-1.8	54538	+H,+Na,+K
Caffeoylquinic acid /isomer	C <sub>16</sub> H <sub>18</sub> O <sub>9</sub>	4.56	353.0873	-1.4	223765	-H	355.1014	-2.6	11899	+H,+Na
3-o-caffeoyl-4-o-sinapoylquinic acid	C <sub>27</sub> H <sub>28</sub> O <sub>13</sub>	5.85	559.1457	0	155297	-H	561.1591	-2.1	15928	+H
Caffeoylquinic acid ethyl ester /isomer	C <sub>18</sub> H <sub>22</sub> O <sub>9</sub>	6.13	381.1187	-1	213130	-H	383.1327	-2.4	23139	+H
Caffeoylquinic acid ethyl ester /isomer	C <sub>18</sub> H <sub>22</sub> O <sub>9</sub>	6.75	381.1188	-0.9	324074	-H	383.1331	-1.6	35625	+H
Caffeoylquinic acid ethyl ester /isomer	C <sub>18</sub> H <sub>22</sub> O <sub>9</sub>	7.03	381.119	-0.3	553767	-H	383.1329	-2.1	96059	+H

**Table 15. Effects of CDLF or CQAF on the viability of MH7A cells after 24 h treatment (*n* = 6)**

Group		Cell viability (%)
Non-treated control	-	100.00±2.65
CQAF	100 µg/mL	101.23±3.15
	200 µg/mL	100.36±2.83
	300 µg/mL	99.89±3.74
	400 µg/mL	99.24±3.39
	500 µg/mL	95.32±0.77*
	600 µg/mL	93.64±0.89**
CDLF	100 µg/mL	100.09±0.68
	200 µg/mL	100.87±1.46
	300 µg/mL	101.29±2.16
	400 µg/mL	99.89±0.96
	500 µg/mL	94.07±1.32**
	600 µg/mL	92.03±1.62**

MH7A cells ( $1 \times 10^4$  cells/well) were seeded in 96-well plates and allowed to adhere for 24 h. The cells were then treated with CDLF or CQAF in doses of 100 µg/mL, 200 µg/mL, 300 µg/mL, 400 µg/mL, 500 µg/mL and 600 µg/mL for 24 h. After treatment, cells were incubated with 10 µL of MTT (Promega, USA) for 2 h, and then the absorbance at 490 nm was measured with a model 680 microplate reader (Bio-rad, USA). The viability was calculated with the following fomula.

$$\text{Cell viability (\%)} = (A_{\text{sample}} - A_{\text{blank}}) / (A_{\text{control}} - A_{\text{blank}}) \times 100\%$$

$A_{\text{sample}}$ : absorbance of CDLF or CQAF group at 490 nm

$A_{\text{control}}$ : absorbance of the none-treated control group at 490 nm

$A_{\text{control}}$ : absorbance of the cultrue medium at 490 nm

The data are presented as the mean ± SD. \* $P < 0.05$ , \*\* $P < 0.01$  when compared with the none-treated control group.



Figure 1. Original western-blot figure of Figure 7 in manuscript. CDFL,  $\beta$ -actin. From left to right: non-treated control group, TNF- $\alpha$  group, CDFL (100  $\mu$ g/mL) group, CDFL (200  $\mu$ g/mL) group, CDFL (400  $\mu$ g/mL) group.



Figure 2. Original western-blot figure of Figure 7 in manuscript. CDFL, COX-2. From left to right: non-treated control group, TNF- $\alpha$  group, CDFL (100  $\mu$ g/mL) group, CDFL (200  $\mu$ g/mL) group, CDFL (400  $\mu$ g/mL) group.



Figure 3. Original western-blot figure of Figure 7 in manuscript. CDFL, iNOS. From left to right: quality control sample, non-treated control group, TNF- $\alpha$  group, CDFL (100  $\mu\text{g/mL}$ ) group, CDFL (200  $\mu\text{g/mL}$ ) group, CDFL (400  $\mu\text{g/mL}$ ) group.

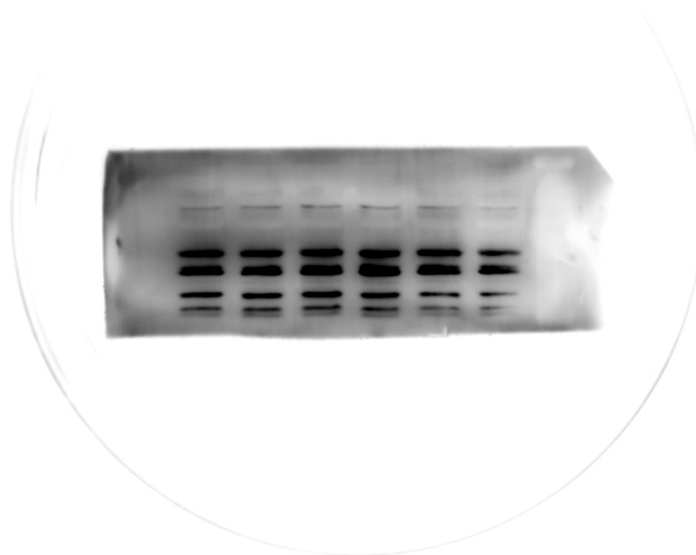


Figure.4 Original western-blot figure of Figure 7 in manuscript. CQAF,  $\beta$ -actin. From left to right: non-treated control group, TNF- $\alpha$  group, CQAF (100  $\mu\text{g/mL}$ ) group, CQAF (200  $\mu\text{g/mL}$ ) group, CQAF (400  $\mu\text{g/mL}$ ) group, quality control sample.

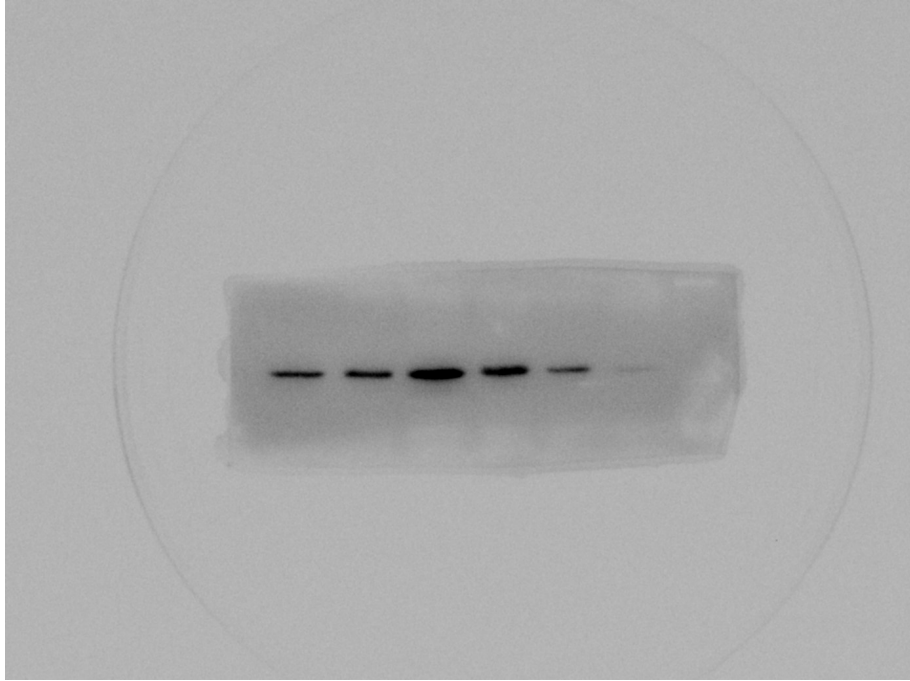


Figure 5. Original western-blot figure of Figure 7 in manuscript. CQAF, COX-2. From left to right: quality control sample, non-treated control group, TNF- $\alpha$  group, CQAF (100  $\mu\text{g/mL}$ ), CQAF (200  $\mu\text{g/mL}$ ), CQAF (400  $\mu\text{g/mL}$ ).

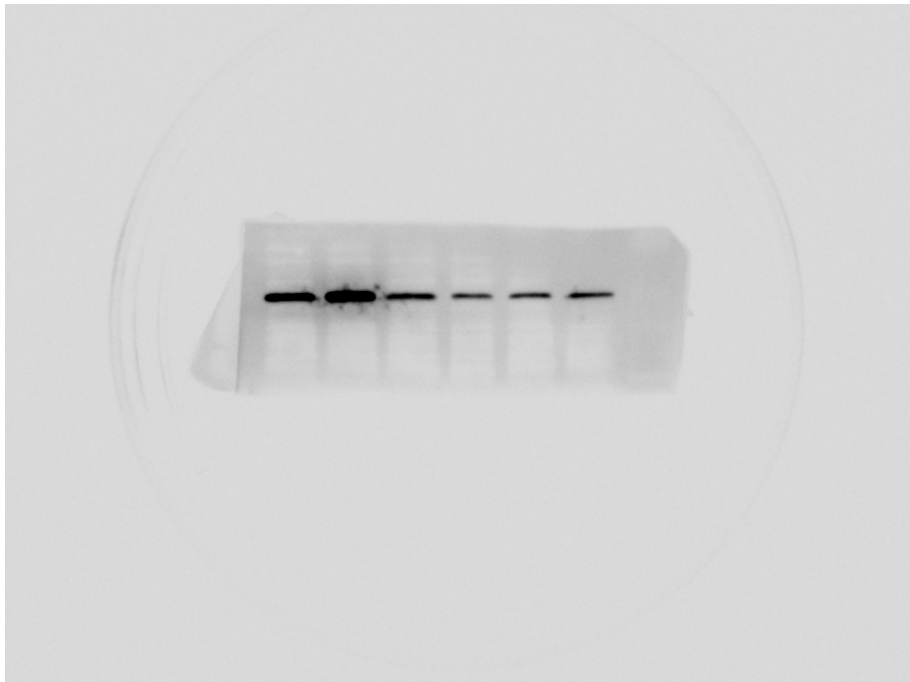


Figure 6. Original western-blot figure of Figure 7 in manuscript. CQAF, iNOS. From left to right: non-treated control group, TNF- $\alpha$  group, CQAF (100  $\mu\text{g/mL}$ ), CQAF (200  $\mu\text{g/mL}$ ), CQAF (400  $\mu\text{g/mL}$ ), quality control sample.



Figure 7. Original western-blot figure of Figure 8 in manuscript. CDFL,  $\beta$ -actin. From left to right: non-treated control group, TNF- $\alpha$  group, CDFL (100  $\mu\text{g/mL}$ ) group, CDFL (200  $\mu\text{g/mL}$ ) group, CDFL (400  $\mu\text{g/mL}$ ) group, quality control sample.

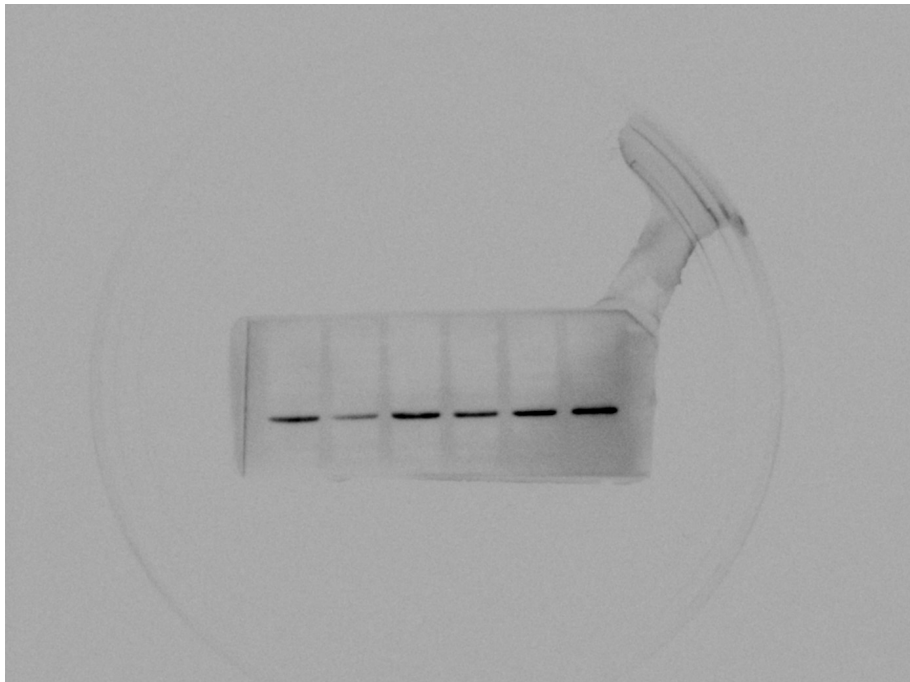


Figure 8. Original western-blot figure of Figure 8 in manuscript. CDFL, I $\kappa$ B $\alpha$ . From left to right: non-treated control group, TNF- $\alpha$  group, CDFL (100  $\mu\text{g/mL}$ ) group, CDFL (200  $\mu\text{g/mL}$ ) group, CDFL (400  $\mu\text{g/mL}$ ) group, quality control sample.

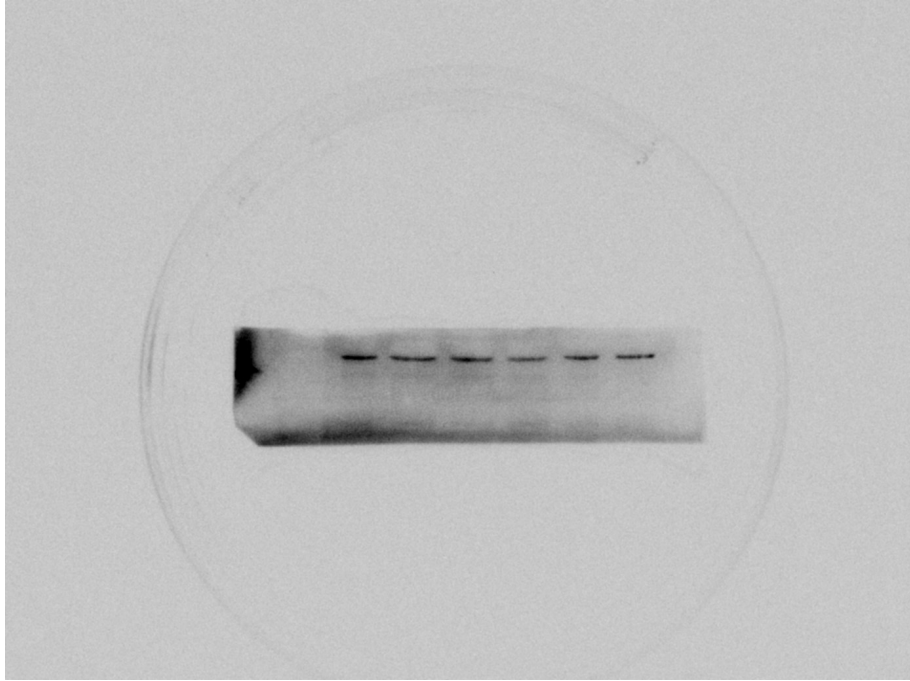


Figure 9. Original western-blot figure of Figure 8 in manuscript. CDFL, p65. From left to right: non-treated control group, TNF- $\alpha$  group, CDFL (100  $\mu\text{g/mL}$ ) group, CDFL (200  $\mu\text{g/mL}$ ) group, CDFL (400  $\mu\text{g/mL}$ ) group, quality control sample.

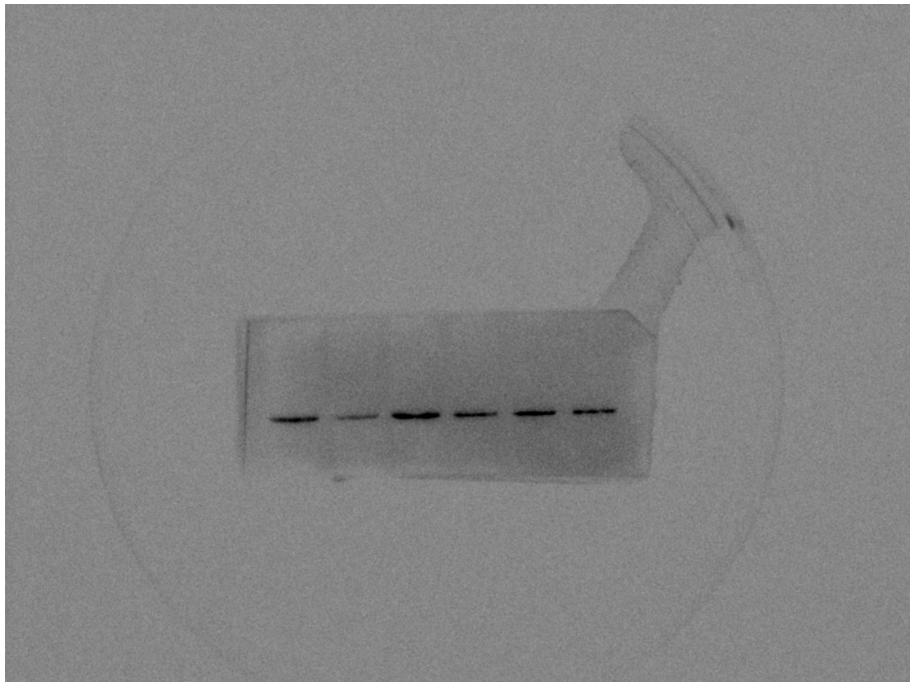


Figure 10. Original western-blot figure of Figure 8 in manuscript. CDFL, p-IkBa. From left to right: quality control sample, non-treated control group, TNF- $\alpha$  group, CDFL (100  $\mu\text{g/mL}$ ) group, CDFL (200  $\mu\text{g/mL}$ ) group, CDFL (400  $\mu\text{g/mL}$ ) group.



Figure.11. Original western-blot figure of Figure 8 in manuscript. CQAF,  $\beta$ -actin. From left to right: non-treated control group, TNF- $\alpha$  group, CQAF (100  $\mu$ g/mL) group, CQAF (200  $\mu$ g/mL) group, CQAF (400  $\mu$ g/mL) group, quality control sample.

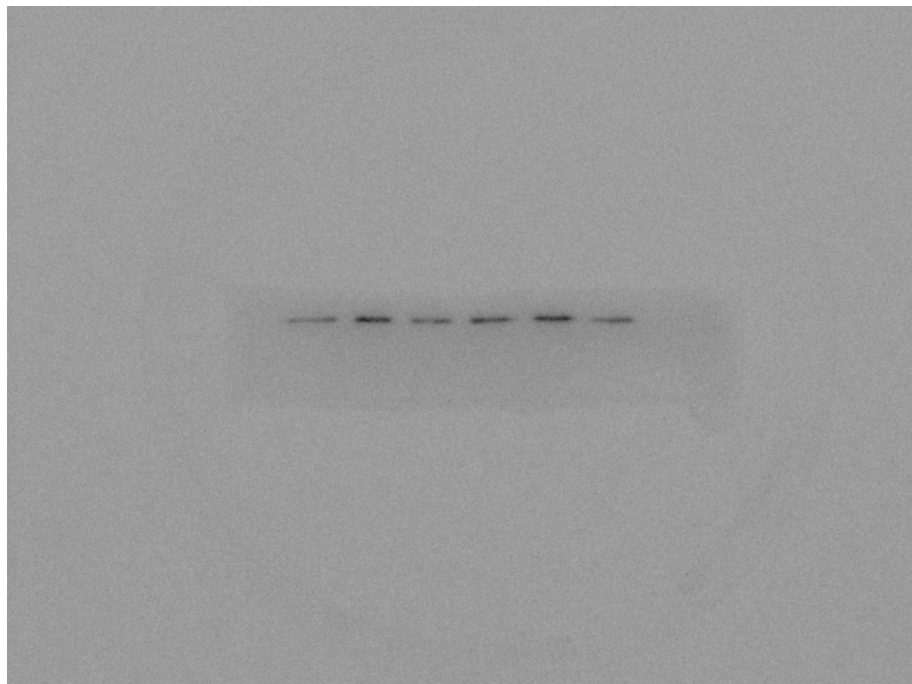


Figure 12. Original western-blot figure of Figure 8 in manuscript. CQAF,  $I\kappa B\alpha$ . From left to right: quality control sample, non-treated control group, TNF- $\alpha$  group, CQAF (100  $\mu$ g/mL) group, CQAF (200  $\mu$ g/mL) group, CQAF (400  $\mu$ g/mL) group.

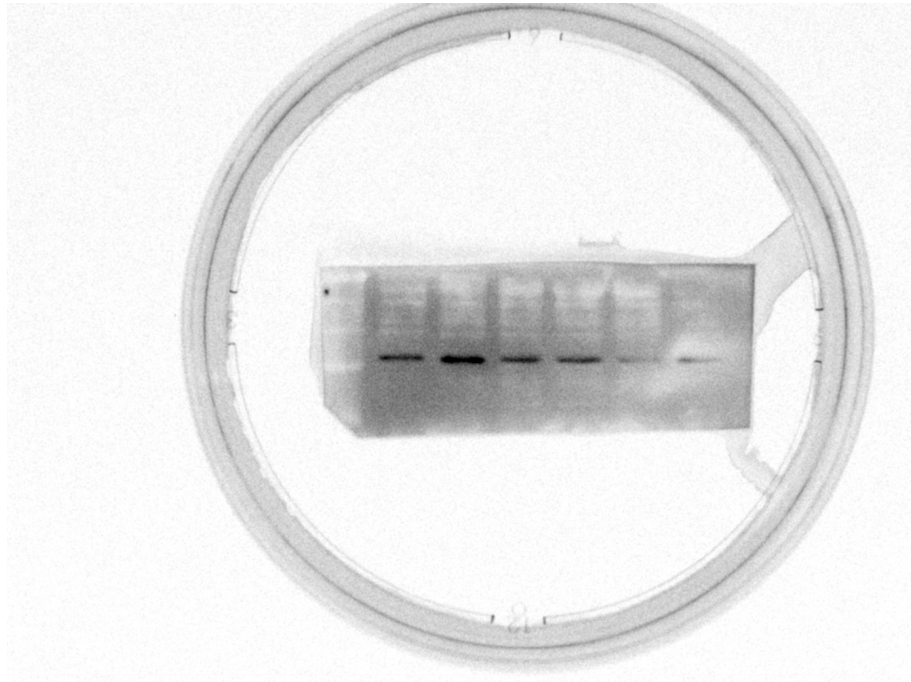


Figure 13. Original western-blot figure of Figure 8 in manuscript. CQAF, p65. From left to right: non-treated control group, TNF- $\alpha$  group, CQAF (100  $\mu\text{g/mL}$ ) group, CQAF (200  $\mu\text{g/mL}$ ) group, CQAF (400  $\mu\text{g/mL}$ ) group, quality control sample.



Figure 14 Original western-blot figure of Figure 8 in manuscript. CQAF, p-IkBa. From left to right: non-treated control group, TNF- $\alpha$  group, CQAF (100  $\mu\text{g/mL}$ ) group, CQAF (200  $\mu\text{g/mL}$ ) group, CQAF (400  $\mu\text{g/mL}$ ) group, quality control sample.



Figure 15. Original western-blot figure of Figure 9 in manuscript. CDLF,  $\beta$ -actin. From left to right: non-treated control group, TNF- $\alpha$  group, CDLF (100  $\mu$ g/mL) group, CDLF (200  $\mu$ g/mL) group, CDLF (400  $\mu$ g/mL) group, quality control sample.

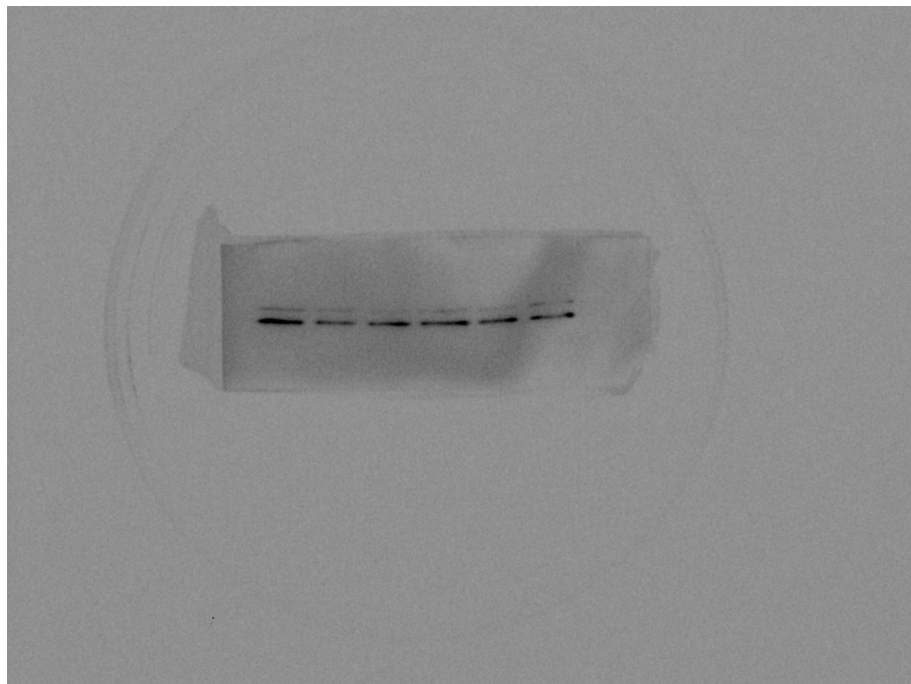


Figure 16. Original western-blot figure of Figure 9 in manuscript. CDLF, ERK<sub>1/2</sub>. From left to right: quality control sample, non-treated control group, TNF- $\alpha$  group, CDLF (100  $\mu$ g/mL) group, CDLF (200  $\mu$ g/mL) group, CDLF (400  $\mu$ g/mL) group.



Figure 17. Original western-blot figure of Figure 9 in manuscript. CDLF, JNK. From left to right: quality control sample, non-treated control group, TNF- $\alpha$  group, CDLF (100  $\mu\text{g/mL}$ ) group, CDLF (200  $\mu\text{g/mL}$ ) group, CDLF (400  $\mu\text{g/mL}$ ) group.



Figure 18. Original western-blot figure of Figure 9 in manuscript. CDLF, p38. From left to right: non-treated control group, TNF- $\alpha$  group, CDLF (100  $\mu\text{g/mL}$ ) group, CDLF (200  $\mu\text{g/mL}$ ) group, CDLF (400  $\mu\text{g/mL}$ ) group.



Figure 19 Original western-blot figure of Figure 9 in manuscript. CDLF, p-ERK<sub>1/2</sub>. From left to right: non-treated control group, TNF- $\alpha$  group, CDLF (100  $\mu$ g/mL) group, CDLF (200  $\mu$ g/mL) group, CDLF (400  $\mu$ g/mL) group, quality control sample.

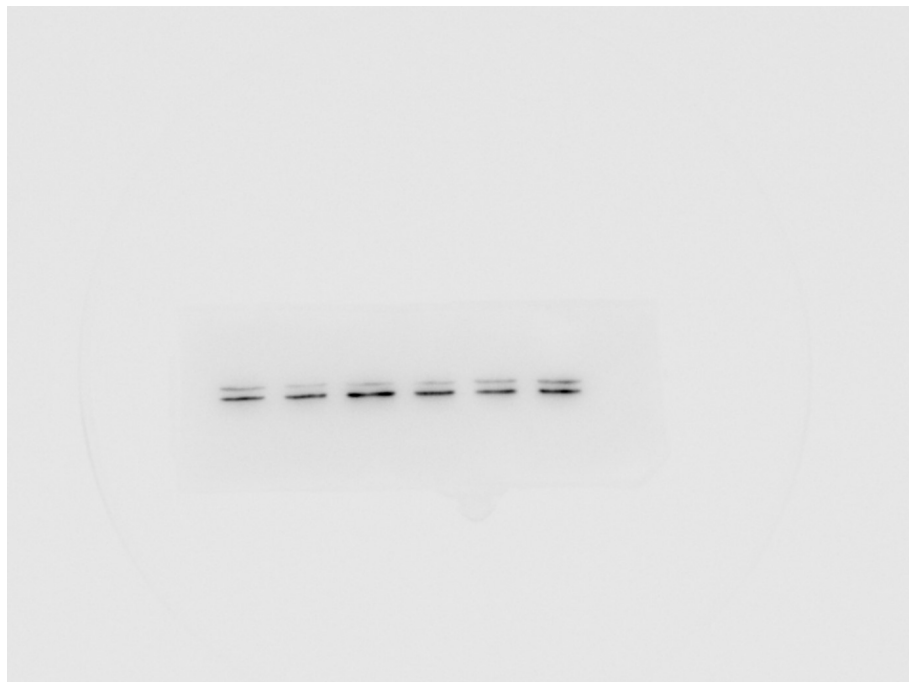


Figure 20. Original western-blot figure of Figure 9 in manuscript. CDLF, p-JNK. From left to right: quality control sample, non-treated control group, TNF- $\alpha$  group, CDLF (100  $\mu$ g/mL) group, CDLF (200  $\mu$ g/mL) group, CDLF (400  $\mu$ g/mL) group.

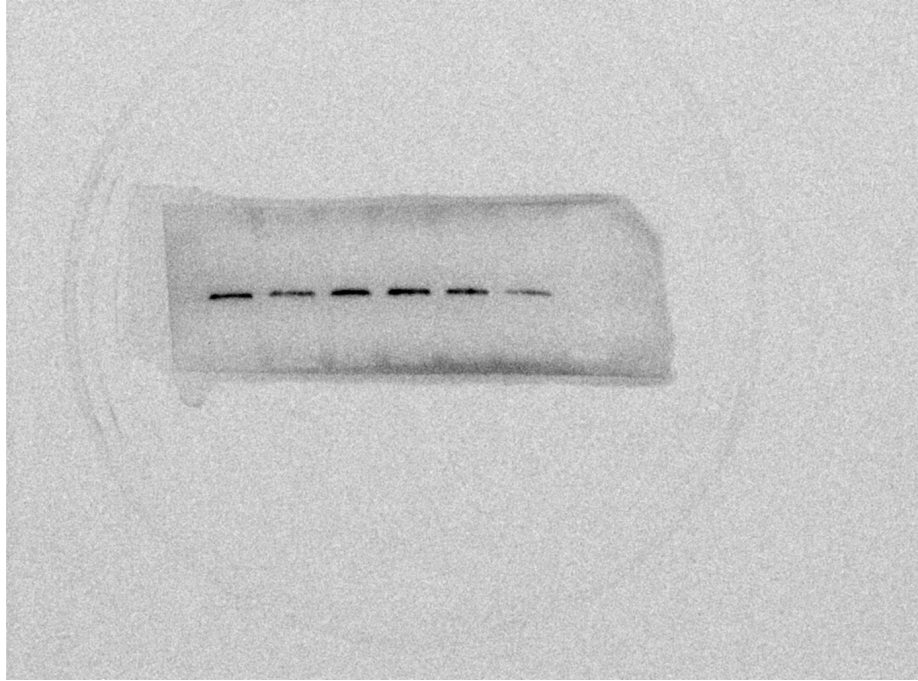


Figure 21. Original western-blot figure of Figure 9 in manuscript. CDLF, p-p38. From left to right: quality control sample, non-treated control group, TNF- $\alpha$  group, CDLF (100  $\mu\text{g/mL}$ ) group, CDLF (200  $\mu\text{g/mL}$ ) group, CDLF (400  $\mu\text{g/mL}$ ) group.



Figure 22. Original western-blot figure of Figure 9 in manuscript. CQAF,  $\beta$ -actin. From left to right: non-treated control group, TNF- $\alpha$  group, CQAF (100  $\mu\text{g/mL}$ ) group, CQAF (200  $\mu\text{g/mL}$ ) group, CQAF (400  $\mu\text{g/mL}$ ) group, quality control sample

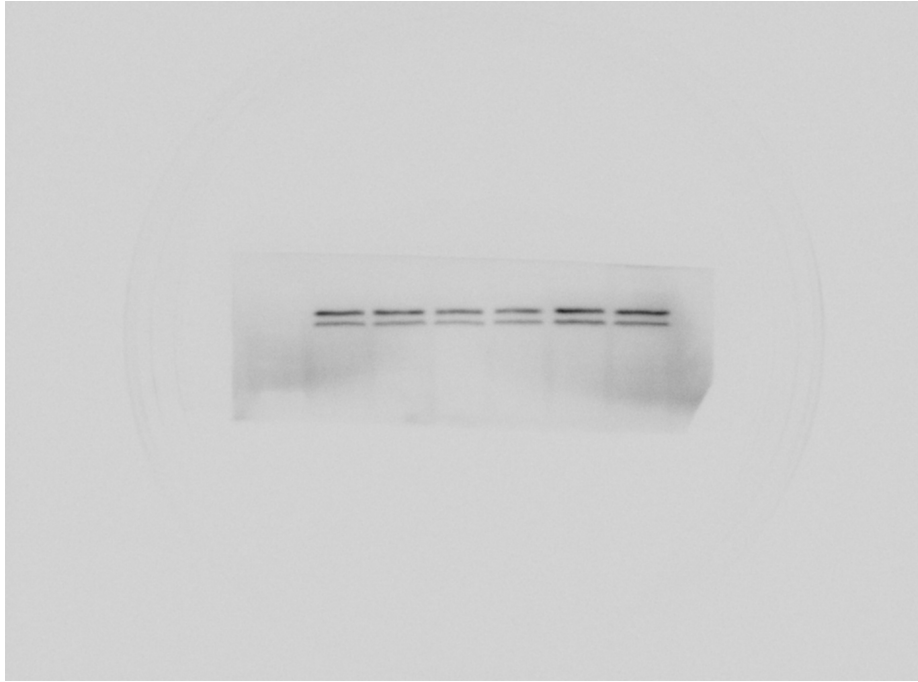


Figure 23. Original western-blot figure of Figure 9 in manuscript. CQAF, ERK<sub>1/2</sub>. From left to right: quality control sample, non-treated control group, TNF- $\alpha$  group, CQAF (100  $\mu$ g/mL) group, CQAF (200  $\mu$ g/mL) group, CQAF (400  $\mu$ g/mL) group.



Figure 24. Original western-blot figure of Figure 9 in manuscript. CQAF, JNK. From left to right: non-treated control group, TNF- $\alpha$  group, CQAF (100  $\mu$ g/mL) group, CQAF (200  $\mu$ g/mL) group, CQAF (400  $\mu$ g/mL) group, quality control sample.

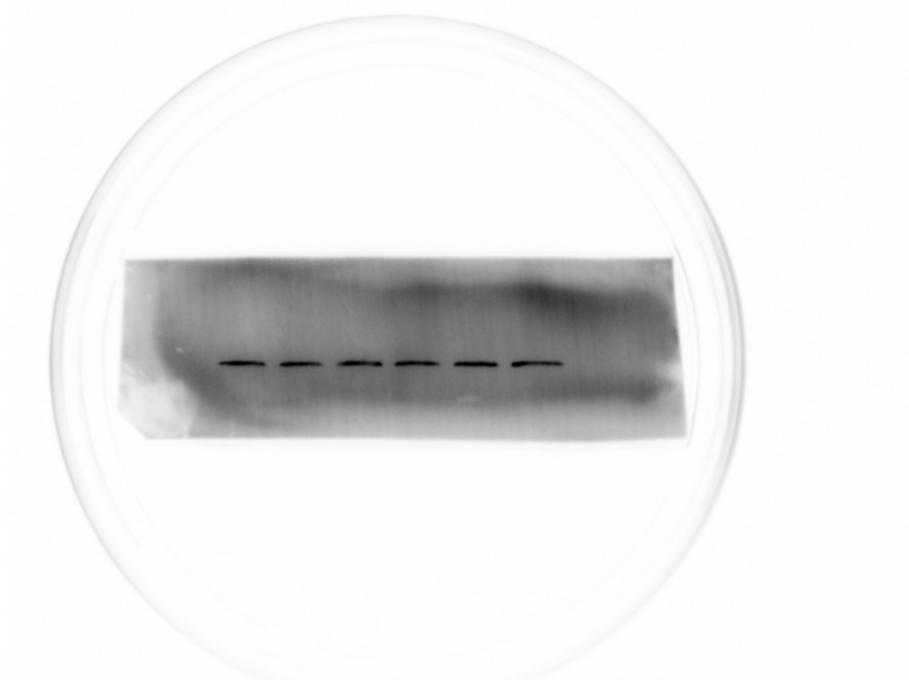


Figure 25. Original western-blot figure of Figure 9 in manuscript. CQAF, p38. From left to right: non-treated control group, TNF- $\alpha$  group, CQAF (100  $\mu\text{g/mL}$ ) group, CQAF (200  $\mu\text{g/mL}$ ) group, CQAF (400  $\mu\text{g/mL}$ ) group, quality control sample.



Figure 26. Original western-blot figure of Figure 9 in manuscript. CQAF, p-ERK<sub>1/2</sub>. From left to right: non-treated control group, TNF- $\alpha$  group, CQAF (100  $\mu\text{g/mL}$ ) group, CQAF (200  $\mu\text{g/mL}$ ) group, CQAF (400  $\mu\text{g/mL}$ ) group, quality control sample.

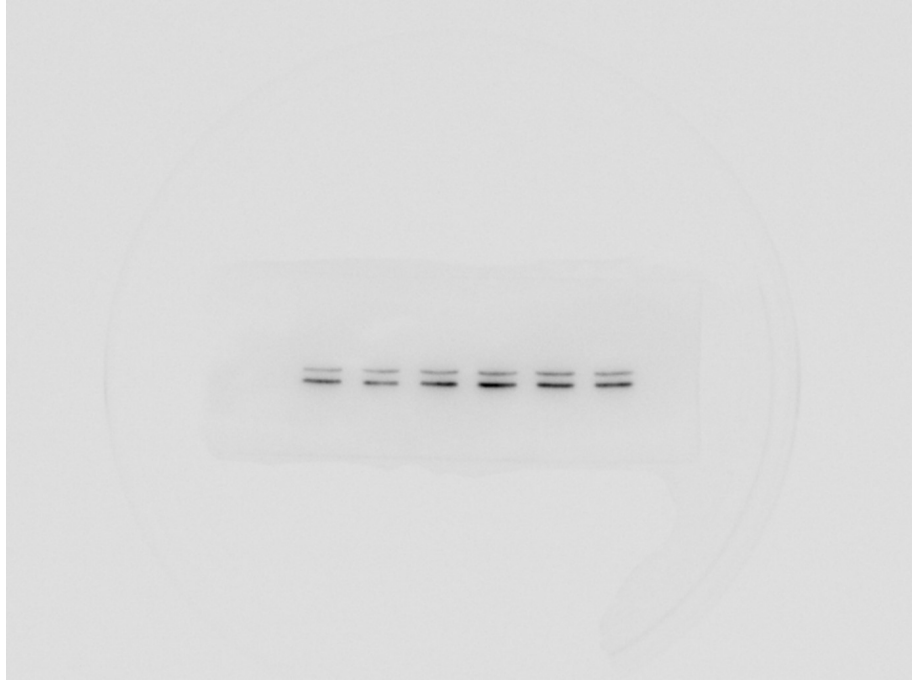


Figure 27. Original western-blot figure of Figure 9 in manuscript. CQAF, p-JNK. From left to right: quality control sampl, non-treated control group, TNF- $\alpha$  group, CQAF (100  $\mu\text{g/mL}$ ) group, CQAF (200  $\mu\text{g/mL}$ ) group, CQAF (400  $\mu\text{g/mL}$ ) group.

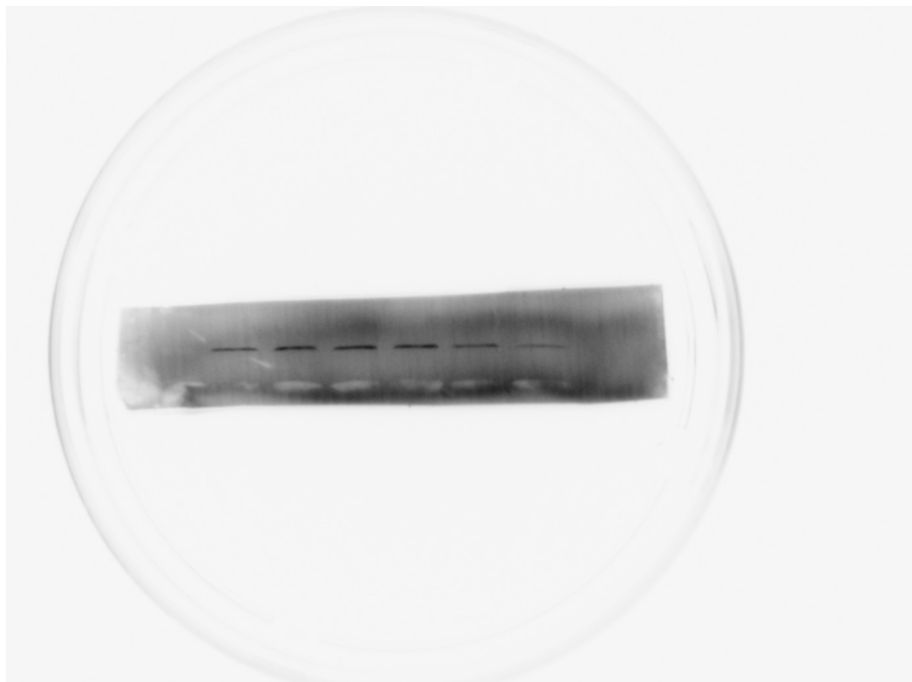


Figure 28. Original western-blot figure of Figure 9 in manuscript. CQAF, p-p38. From left to right: non-treated control group, TNF- $\alpha$  group, CQAF (100  $\mu\text{g/mL}$ ) group, CQAF (200  $\mu\text{g/mL}$ ) group, CQAF (400  $\mu\text{g/mL}$ ) group, quality control sample.



Figure 29. Whole plant of *Periploca forrestii* used in the present study.



Figure 30. Stalks of *Periploca forrestii* used in the present study.



Figure 31. Roots and stalks of *Periploca forrestii* used in the present study.

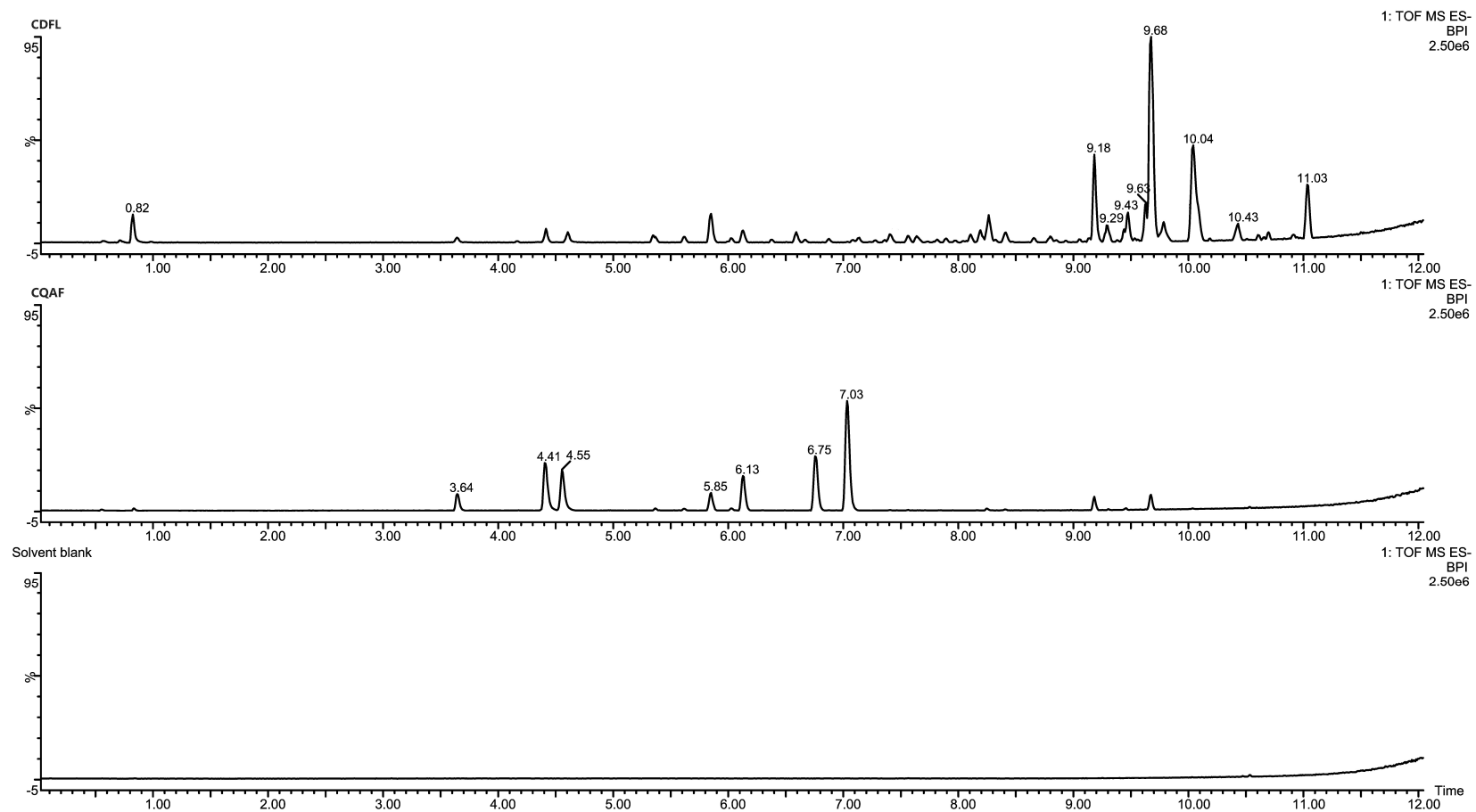


Figure 32. UPLC-QTOF-MS chromatograms of CDFL (0.5 mg/mL) and CQAF (0.05 mg/mL). Negative ion mode.

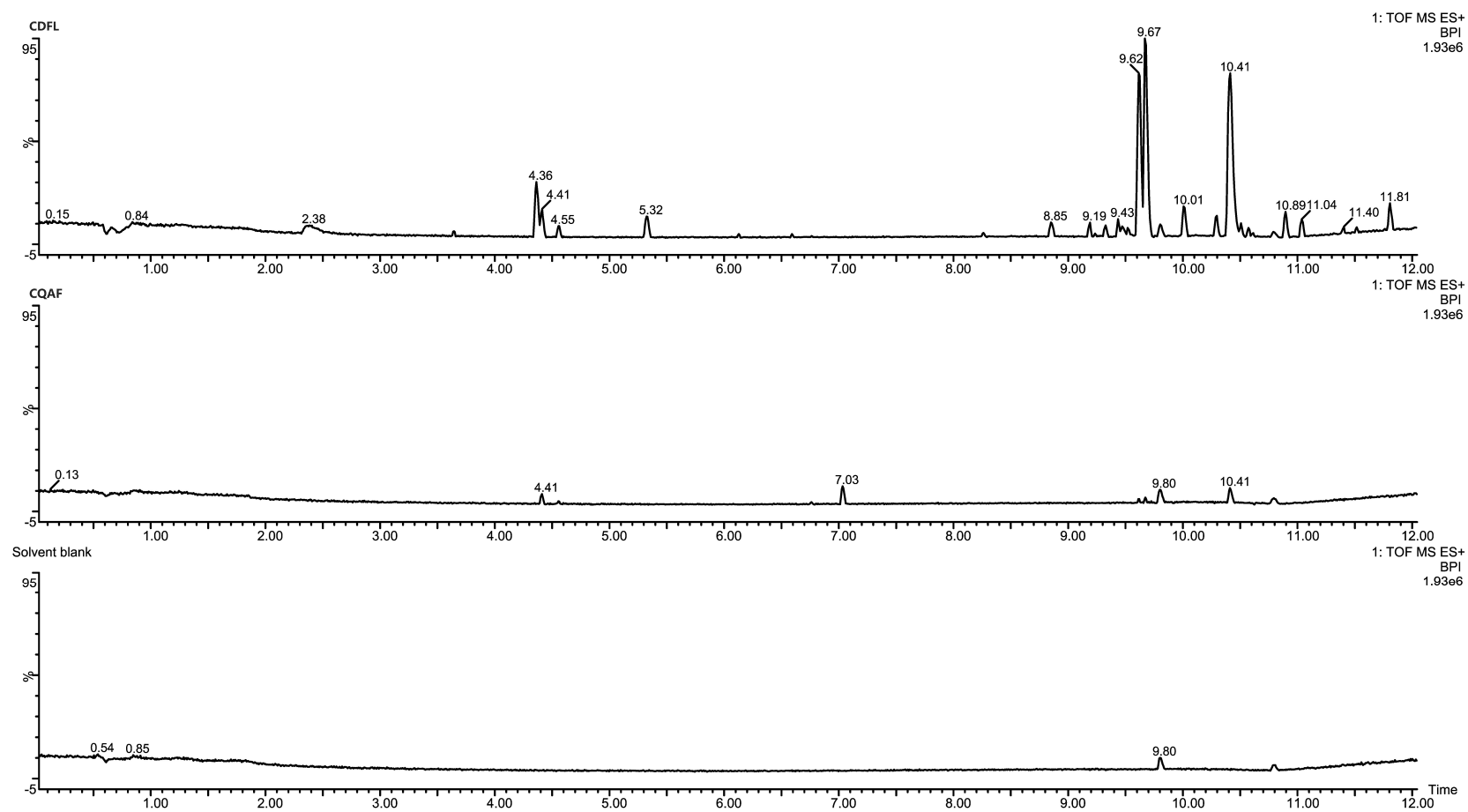


Figure 33. UPLC-QTOF-MS chromatograms of CDFL (0.5 mg/mL) and CQAF (0.05 mg/mL). Positive ion mode.