Electronic Supplementary Information (ESI)

Therapeutic deep eutectic solvent-based microemulsion enhances anti-inflammatory efficacy of curcuminoids and aromatic-turmerone extracted from *Curcuma longa* L.

Nassareen Supaweera[a], Wanatsanan Chulrik[b], Chutima Jansakun[b], Phuangthip Bhoopong[b,d] Gorawit Yusakul[c*], Warangkana Chunglok[b,d*]

[a]Health Sciences (International Program), College of Graduate Studies, Walailak University, Nakhon Si Thammarat 80160, Thailand

[b]School of Allied Health Sciences, Walailak University, Nakhon Si Thammarat 80160, Thailand

[c]School of Pharmacy, Walailak University, Nakhon Si Thammarat 80160, Thailand

[d]Food Technology and Innovation Research Center of Excellence, Research and Innovation Institute of Excellence, Walailak University, Nakhon Si Thammarat, Thailand

***Corresponding author**

Dr. Warangkana Chunglok

School of Allied Health Sciences, Walailak University, Nakhon Si Thammarat 80160, Thailand

E-mail: cwarang@wu.ac.th and aon.cwarang@gmail.com

Dr. Gorawit Yusakul

School of Pharmacy, Walailak University, Nakhon Si Thammarat 80160, Thailand

E-mail: gorawit.yu@wu.ac.th and gorawit.yu@mail.wu.ac.th

Table of Contents

Table S1. HDES microemulsions after dilution in water (1/1000) (n = 3, mean ± SEM)

	Components (wt%)			Blank microemulsion		Microemulsion extracts	
Microemulsion	HDES	Smix	Water	Size (nm)	PDI	Size (nm)	PDI
HDES of octanoic acid:menthol (70:30, mass ratio)							
ME-71	5	90	5	6.20±1.16	0.46±0.11	8.62±1.80	0.50±0.04
ME-72	15	80	5	2.85±0.16	0.40±0.06	4.18±0.14	0.38±0.04
ME-73	25	70	5	7.43±0.69	0.69±0.03	1.38±0.07	0.72±0.02
ME-74	35	60	5	22.7±3.7	0.38±0.08	8.31±0.89	0.49±0.02
HDES of octanoic acid:menthol (40:60, mass ratio)							
ME-41	5	90	5	6.63±1.74	0.46±0.07	3.42±0.57	0.32±0.03
ME-42	15	80	5	1.21±0.75	0.35±0.13	3.40±0.06	0.30±0.00
ME-43	25	70	5	8.43±0.23	0.54±0.02	13.6±0.1	0.63±0.01
ME-44	35	60	5	5.82±0.21	0.41±0.02	9.94±1.13	0.61±0.05
HDES of octanoic acid:menthol (20:80, mass ratio)							
ME-21	5	90	5	7.08±1.83	0.54±0.06	5.50±0.55	0.64±0.09
ME-22	15	80	5	10.4±0.2	0.66±0.01	1.96±0.33	0.21±0.03
ME-23	25	70	5	10.2±0.4	0.65±0.03	18.6±0.1	0.63±0.01
ME-24	35	60	5	12.1±0.2	0.80±0.04	3.02±0.04	0.46±0.01
ME-25	45	50	5	12.7±1.0	0.71±0.02	51.3±6.5	0.90±0.10

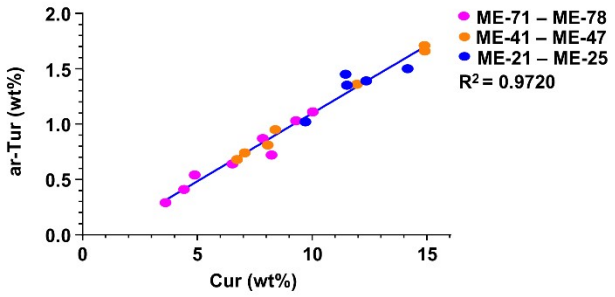

Figure S1. Correlation of extraction yields of Cur and *ar*-Tur

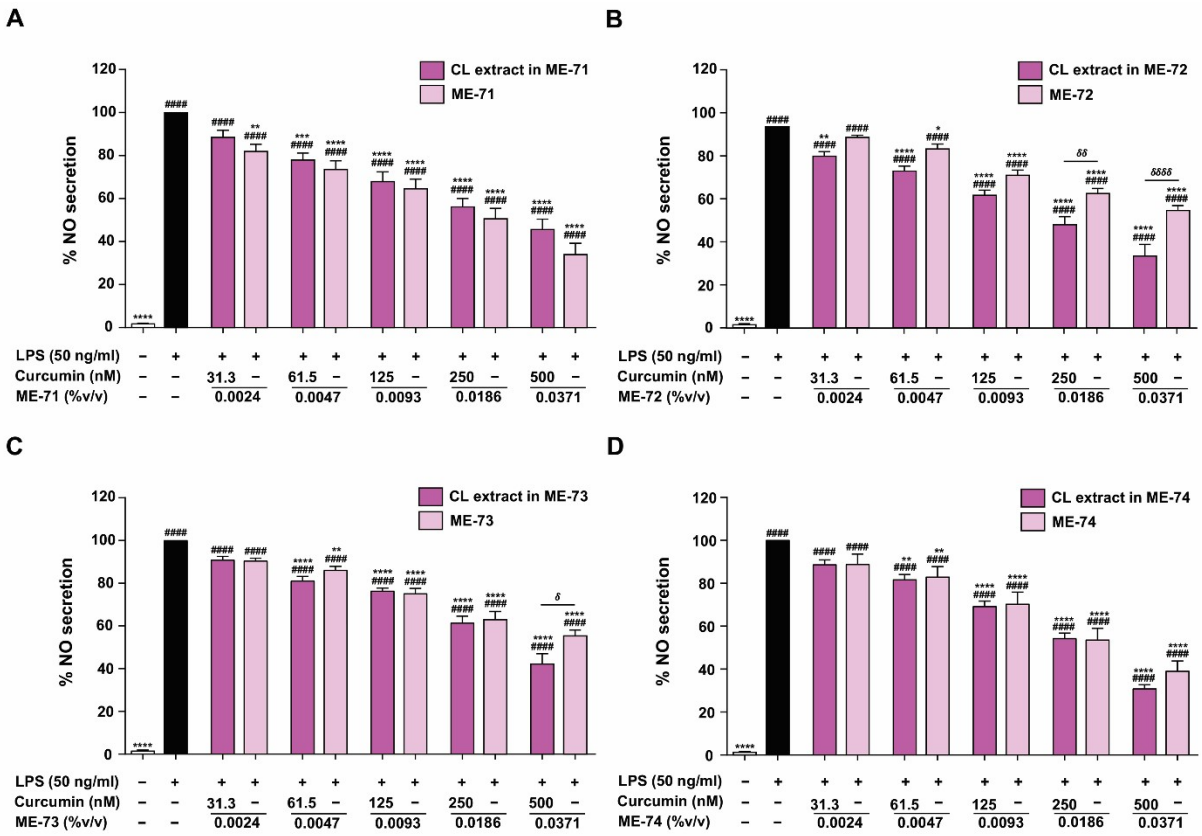

Figure S2. NO inhibition in LPS-activated murine macrophages by CL extract in ME-71 (A), CL extract in ME-72 (B), CL extract in ME-73 (C), and CL extract in ME-74 (D). Data are presented as mean ± SEM of three independent experiments in triplicate. ####$p < 0.0001$ vs. untreated control; *$p < 0.05$, **$p < 0.01$, ****$p < 0.0001$ vs. LPS-stimulated cells; δ$p < 0.05$, δδ$p < 0.01$, and δδδδ$p < 0.0001$ vs. ME-71, ME-72, ME-73, or ME-74.

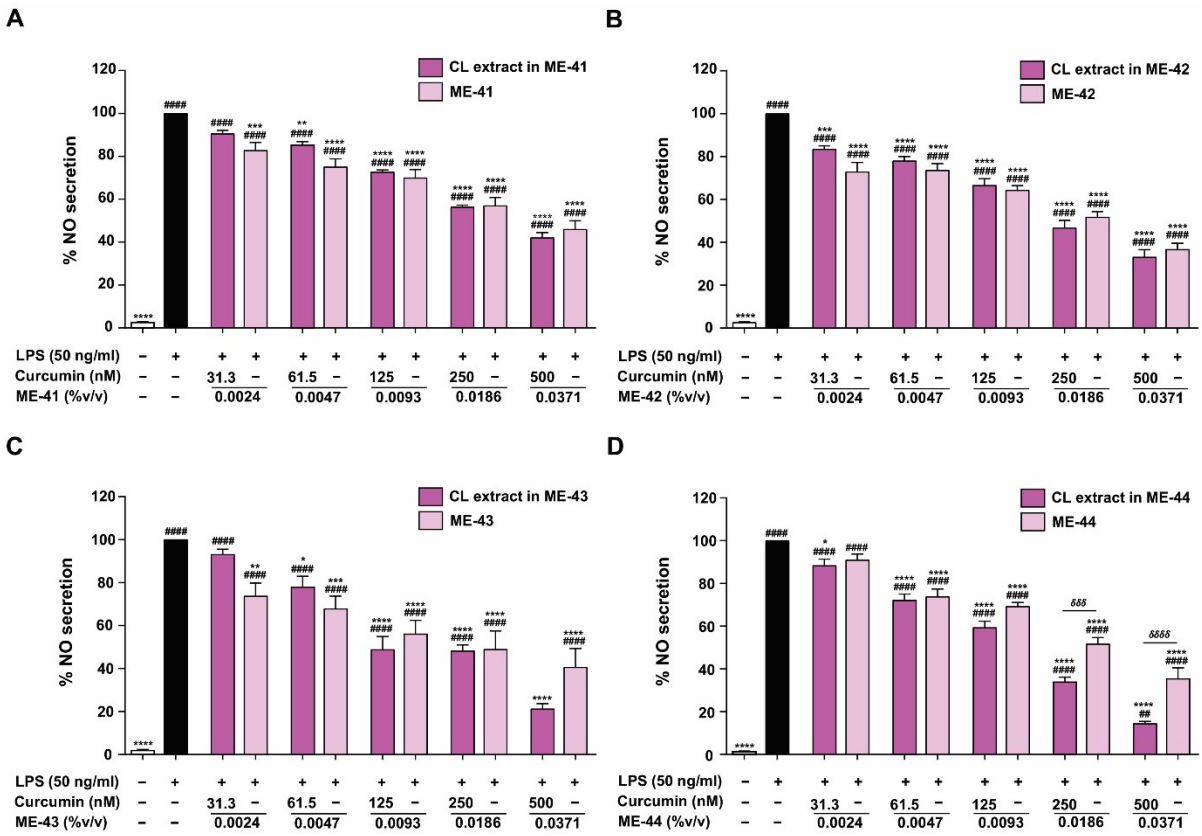

Figure S3. NO inhibition in LPS-activated murine macrophages by CL extract in ME-41 (A), CL extract in ME-42 (B), CL extract in ME-43(C), and CL extract in ME-44 (D). Data are presented as mean ± SEM of three independent experiments in triplicate. ## $p < 0.01$, ####$p < 0.0001$ vs. untreated control; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$ vs. LPS-stimulated cells; δδδ$p < 0.001$, δδδδ$p < 0.0001$ vs. ME-41, ME-42, ME-43, or ME-44.

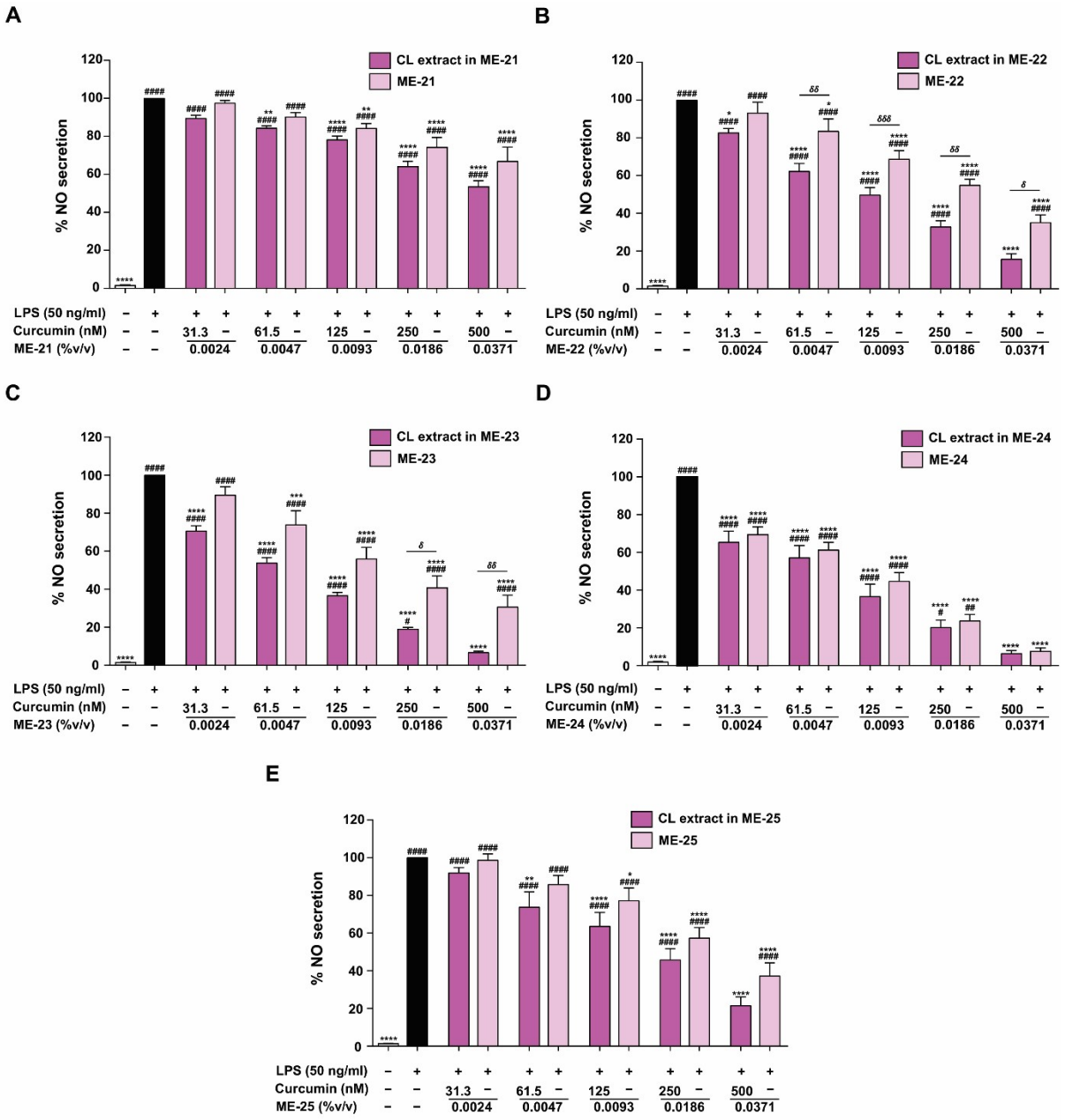

Figure S4. NO inhibition in LPS-activated murine macrophages by CL extract in ME-21 (A), CL extract in ME-22 (B), CL extract in ME-23 (C), CL extract in ME-24 (D), and CL extract in ME-25 (E). Data are presented as mean ± SEM of three independent experiments in triplicate. #$p < 0.05$, ## $p < 0.01$, ####$p < 0.0001$ vs. untreated control; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$ vs. LPS-stimulated cells; δ$p < 0.05$, δδ$p < 0.01$, and δδδ$p < 0.001$ vs. ME-21, ME-22, ME-23, ME-24, or ME-25.

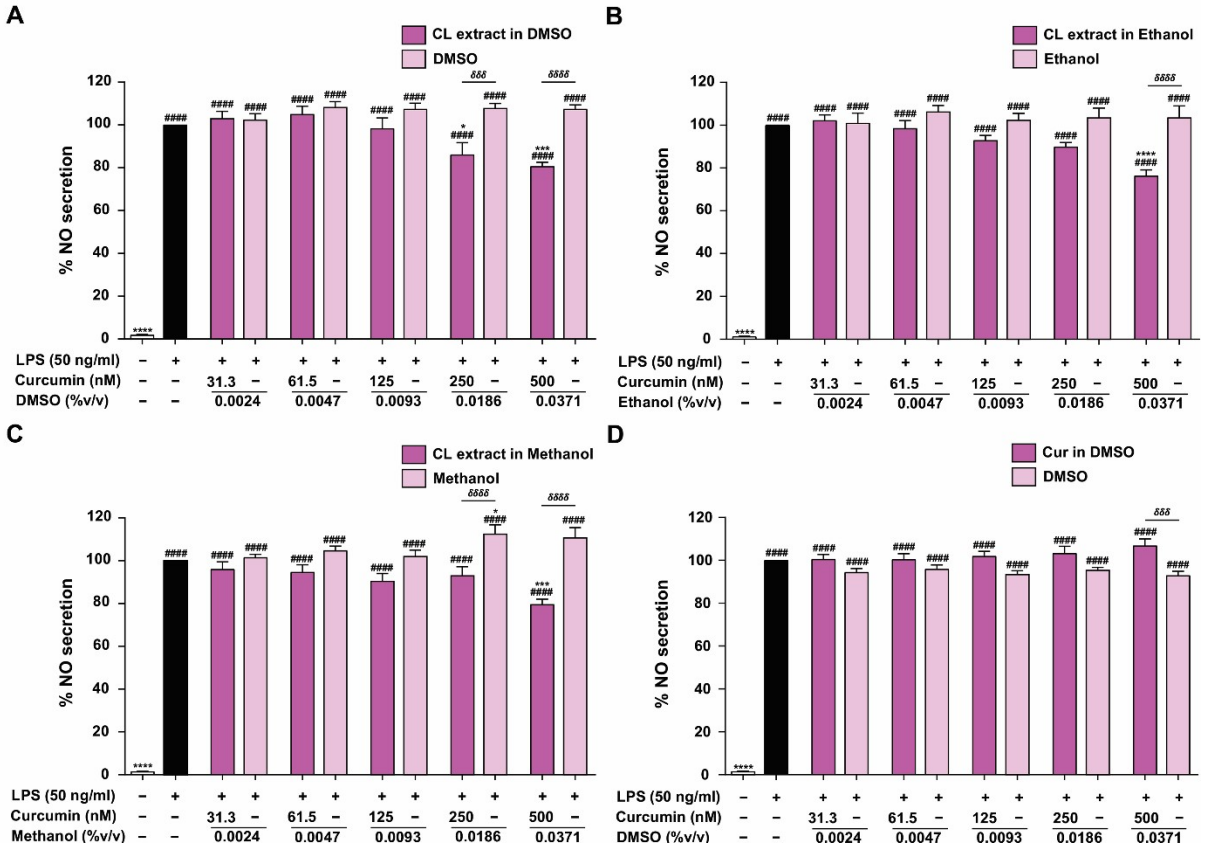

Figure S5. NO inhibition in LPS-activated murine macrophages by CL extract in DMSO (A), CL extract in ethanol (B), CL extract in methanol (C), and Cur in DMSO (D). Data are presented as mean ± SEM of three independent experiments in triplicate. ####$p < 0.0001$ vs. untreated control; *$p < 0.05$, ***$p < 0.001$, ****$p < 0.0001$ vs. LPS-stimulated cells; δδδ$p < 0.001$, δδδδ$p < 0.0001$ vs. DMSO, ethanol, or methanol

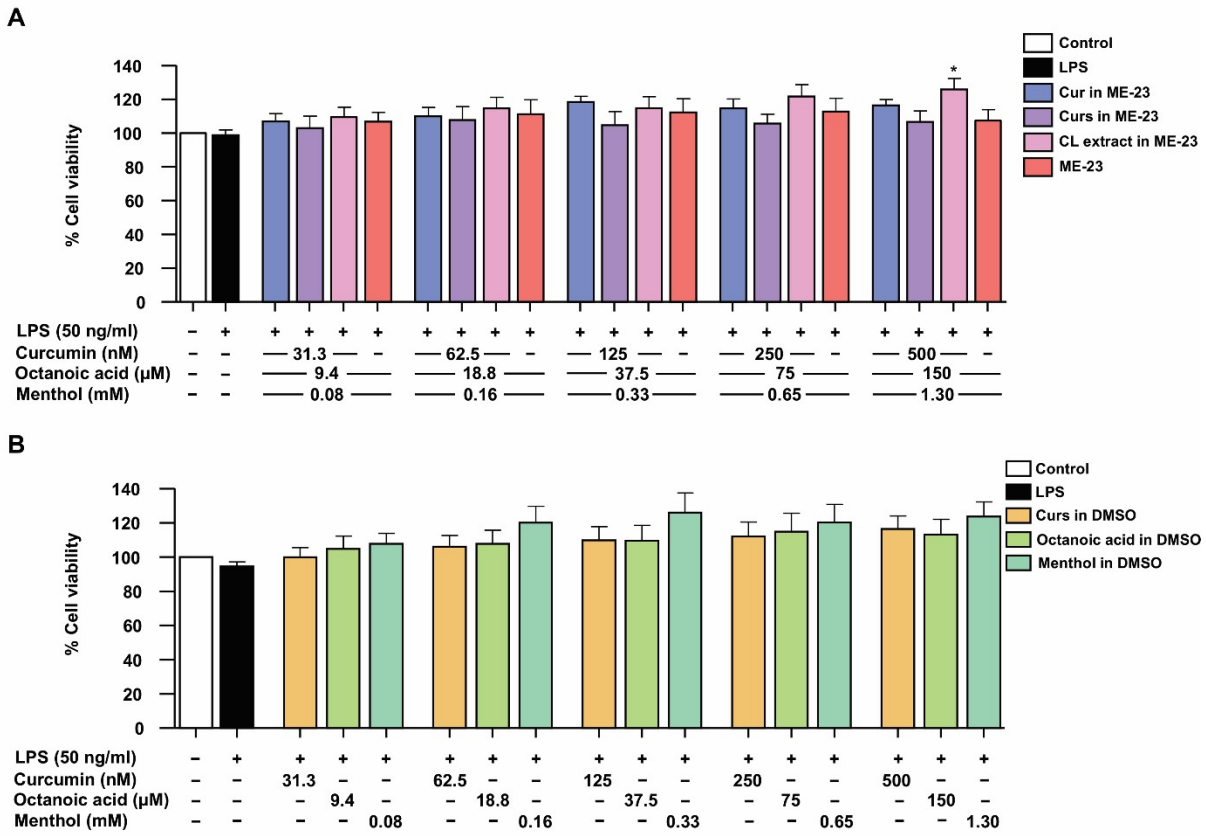

Figure S6. Cell viability in LPS-activated murine macrophage by Cur in ME-23, Curs in ME-23, CL extract in ME-23, and ME-23 (A), as well as Curs, octanoic acid, and menthol in DMSO (B). Data are presented as mean ± SEM of three independent experiments in triplicate. *$p < 0.05$ vs. LPS-stimulated cells.

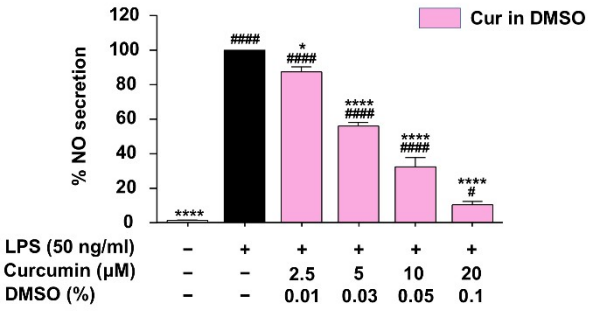

Figure S7. NO inhibition in LPS-activated murine macrophages by Cur in DMSO. Data are presented as mean ± SEM of three independent experiments in triplicate. #$p < 0.05$, ####$p < 0.0001$ vs. untreated control; *$p < 0.05$, ****$p < 0.0001$ vs. LPS-stimulated cells.

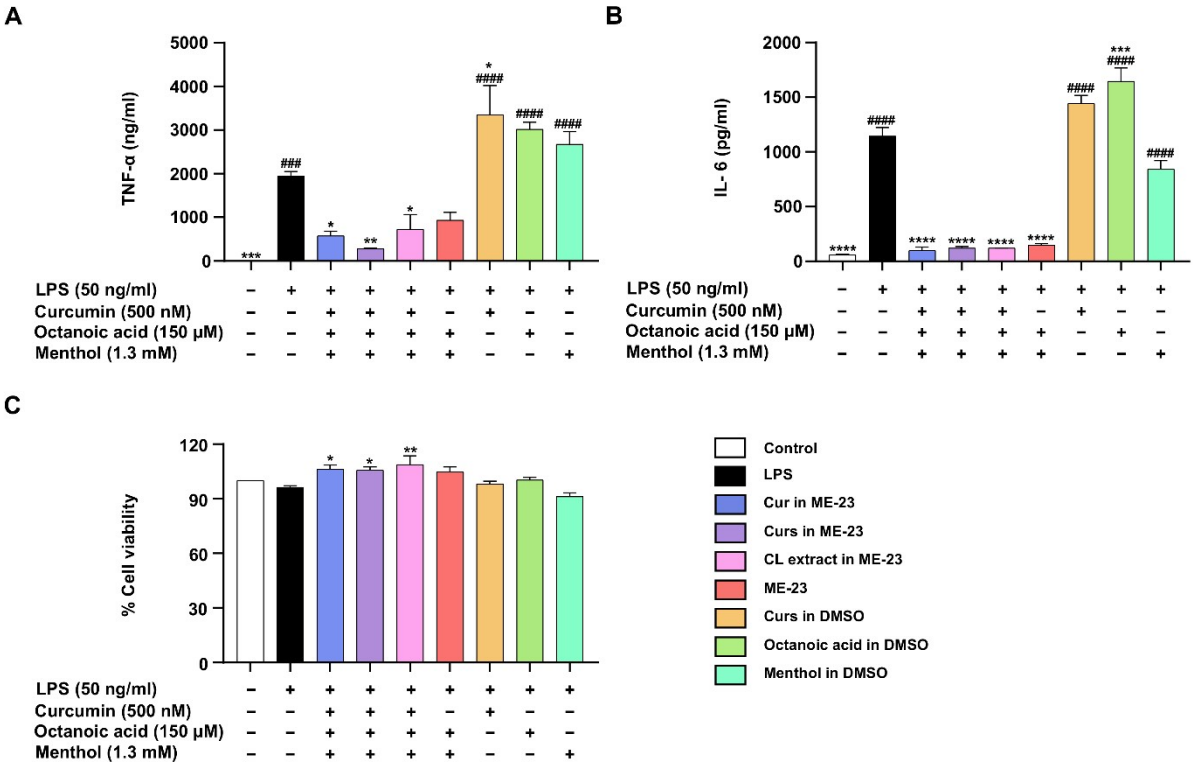

Figure S8. Inflammatory cytokine production and cell viability in LPS-activated differentiated THP-1 macrophages. Anti-inflammatory effects of Cur in ME-23, Curs in ME-23, CL extract in ME-23, and ME-23, as well as Curs, octanoic acid, and menthol in DMSO against inflammatory cytokines TNF-α (A) and IL-6 (B) production in LPS-activated differentiated THP-1 macrophages. (C) Cell viability determined by the MTT assay. Data are presented as mean ± SEM of three independent experiments. ###$p < 0.001$, ####$p < 0.0001$ vs. untreated control; *$p < 0.05$, **$p < 0.01$ ***$p < 0.001$, ****$p < 0.0001$ vs. LPS-stimulated cells.