Supplementary file 1

Phytochemical identification

To identify the individual components of *M. kobus* DC leaf ethanol extract (MLEE), an Agilent 1260 Infinity Series (Agilent Technologies, Santa Clara, CA, USA) equipped with a diode array detector (DAD) system (Agilent Technologies) was used. Chromatographic separation was performed using a Capcell Pak C18 column (5 μm × 4.6 mm × 250 mm, Shiseido, Japan) and an Agilent 6120 single quadrupole at 37°C. Chromatography was performed at room temperature at a flow rate of 1 mL/min, and 10 μL was analyzed for 120 min. The mobile phase consisted of 0.1% formic acid (A) and acetonitrile (B) in a ratio specified by the following binary gradient with linear interpolation: 0 min 20% B, 60 min 30% B, 70 min 60% B, 100 min 70% B, and 120 min 20% B. UV spectra were collected by DAD every 0.4 s from 190 to 750 nm with a resolution of 2 nm. The mass spectrometry parameters were set using Mass Hunter Qualitative Analysis Software Version B.06.00 (Agilent Technologies). The raw data were processed based on retention time and characteristic behavior of MS, including the exact mass, quasi-molecular ions, and in-source fragmentation. The data were compared with those of known compounds in an in-house plant database and the existing literature. The acquisition was performed from 100 to 1700 m/z, with three scans per second.

Cell viability

MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was used to assess the cell viability. RAW 264.7 cells (5×10^4 cells/96-well plates) were pretreated with various concentrations of MLEE (1, 10, 100, or 1000 μg/mL) for 24 h. To evaluate the protective effects of hydrogen peroxide (H_2O_2) inducing oxidative stress, RAW 264.7 cells were pretreated with

MLEE (1, 10, or 100 μg/mL) for 1 h before applying oxidative damage (400 μM) for 24 h. After 24 h of incubation, MTT solution (1 mg/mL) was applied to each well of the plate, and the plate was then incubated for 2 h. To dissolve the formed formazan crystals, 100 μL of dimethyl sulfoxide was applied to each well. Absorbance measurements were then performed on the 96-well plate using a VersaMax™ Microplate Reader (Biocompare, Billerica, MA, USA) at 570 nm. The relative cell viability was presented as a percentage compared to untreated control cells (100%).

qPCR

RAW 264.7 cells (5×10^5 cells/24-well plate) were pretreated with MLEE (1, 10, or 100 μg/mL) or Dexa (50 μg/mL) for 1 h and then stimulated with LPS (1 μg/mL). The superior lobe of the right lung tissue was homogenized using a TissueLyser II (Qiagen, Hilden, Germany). To obtain total RNA from LPS-stimulated RAW 264.7 cells and lung tissues, RNA was extracted using an RNAiso Plus Kit (Takara Bio, Shiga, Japan), and the RNA concentration was quantified using a NanoDrop 2000 spectrophotometer (ThermoFisher scientific, Wilmington, MA, USA). RNA was synthesized as complementary DNA (cDNA) using a RevertAid RT Reverse Transcription Kit (ThermoFisher Scientific, Waltham, MA, USA) at 65 °C for 5 min, at 42 °C for 60 min, and at 70 °C for 5 min. The reaction mixture entering each well of the PCR reaction plate contained 1 μL of cDNA (200 ng, RAW 264.7 cells; 250 ng, lung tissues), 1 μL of forward and reverse primers (0.4 μM), 10 μL of QGreenBlue Master Mix High ROX (QBHR-05 2X, ThermoFisher Scientific), and 8 μL of nuclease-free water. Next, PCR was performed with a StepOnePlus™ Real-Time PCR system (Life Technologies Co., Kallang Avenue, Singapore) according to the manufacturer's instructions. The primer sequences for qPCR are shown in Table S1. qPCR analysis was normalized to GAPDH for RAW 264.7 cells and lung tissues, and the relative quantitation in the expression of target genes was calculated

using the 2−ΔΔCq method in StepOnePlus PCR system software (ThermoFisher Scientific).

ELISA

RAW 264.7 cells (5×10^5 cells/24-well plate) were pretreated with MLEE (1, 10, or 100 μg/mL) or Dexa (50 μg/mL) followed by activation with LPS (1 μg/mL) for 24 h. The supernatant was collected and centrifuged at 1,000 × g for 5 min at 4°C. The protein levels of TNF-α (BD Biosciences, Oxford, UK), IL-1β (Invitrogen, Frederick, MD, USA), and IL-6 (BD Biosciences) from the supernatant of LPS-stimulated RAW 264.7 cells were measured using an ELISA kit. Mouse serum was measured for TNF-α, IL-6, and IgG2a (BD Biosciences, Oxford, UK) using an ELISA kit, and superoxide dismutase (SOD) inhibition was measured using an SOD kit (Sigma-Aldrich, St Louis, MO, USA) following the manufacturer's protocol. Absorbance was calculated and analyzed using SoftMax Pro software version 6 (Biocompare, Billerica, MA, USA) and a spectrophotometer (VersaMax™ Microplate Reader) at 450 nm.

Western blotting

RAW 264.7 cells (5×10^5 cells/24-well plate) were pretreated with MLEE (100 μg/mL) or Dexa (50 μg/mL) for 1 h and then stimulated with LPS (1 μg/mL) for 24 h. The medium was removed, washed twice with cold PBS, and the cells were gathered in 100 μL of RIPA buffer (Biosesang, Seongnam, South Korea) containing a protease/phosphatase inhibitor cocktail (Roche, Mannheim, Germany). The inferior lobe of the right lung tissue was homogenized using a TissueLyser II (Qiagen) with RIPA buffer containing a protease/phosphatase inhibitor cocktail. After sonication for 30 s, the samples were left on ice for 20 min. Cell lysates were centrifuged at 16,000 × g at 4 °C for 20 min to obtain total protein. All proteins were measured for absorbance at 595 nm using the Bradford Protein Assay Kit (Bio-Rad Laboratories, Hercules, CA, USA) for quantification. Samples were electrophoresed on a 7.5% (COX-2 and

iNOS for in vitro) or 12.5% (Nrf2, COX-2 and NFκB for in vivo) sodium dodecyl sulfate (SDS)-polyacrylamide gel and then transferred onto a nitrocellulose membrane (Pall Life Science, Port Washington, NY, USA). The membrane was blocked with 5% bovine serum albumin in Tris-buffered saline containing 0.5% Tween 20 (TBST). To recognize specific proteins, the membrane was incubated overnight at 4 °C with the appropriate antibodies: iNOS (Santa Cruz, SC651, 130 kDa, 1:500), COX-2 (CST, #4842, 74 kDa, 1:2000), Nrf2 (Abcam, #PA5-88084, 110 kDa, 1:1000), NF-κB p65 (Cruz, SC8008, 65 kDa, 1:1000), and β-actin (as loading control, Invitrogen, 43 kDa, MA5-15739, 1:3000). Target proteins were detected in the membrane using a SuperSignal West Pico Chemiluminescent Substrate (Thermo Fisher Scientific) in G:BOX Chemi XRQ (Syngene, Cambridge, UK).

Supplementary figures and table

Figure S1. Extraction of *Magnolia kobus* DC and cell viability on RAW 264.7 cell. (A) A photograph showing *Magnolia kobus* DC leaves on a petri dish. (B) A process flow scheme presenting the extraction of *Magnolia kobus* DC for MLEE. (C) Macrophages viability was measured using MTT assay to determine the effect of MLEE. Data sets is displayed as the mean ± SEM (n = 5). $^{*}p < 0.05$ compared with untreated control group. MLEE: *Magnolia kobus* DC leaf ethanol extract.

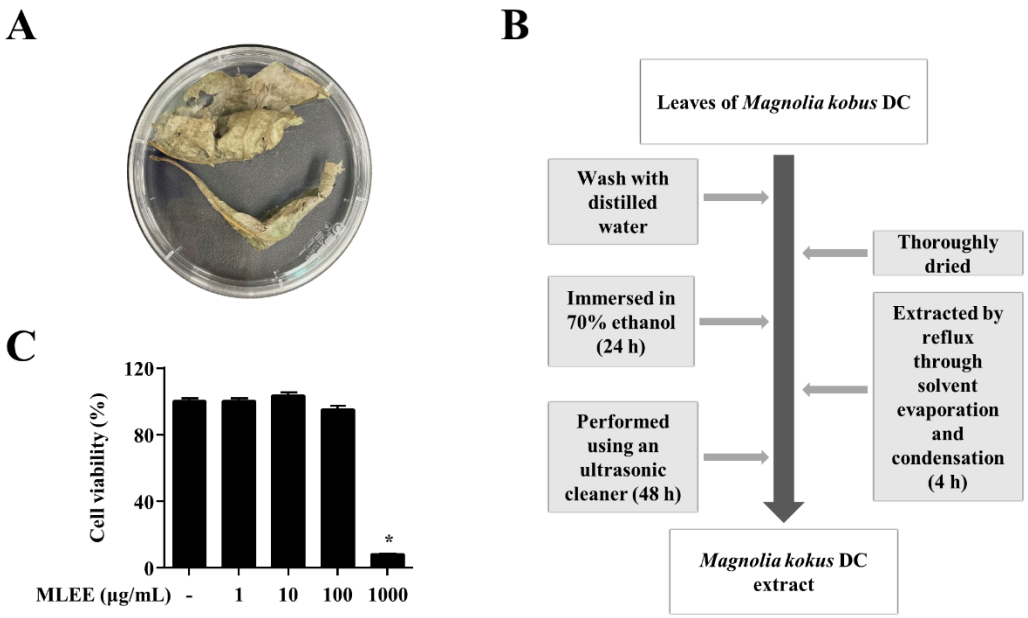

Table S1. Primer pair sequences used in quantitative polymerase chain reaction.

Mouse			
Primer	**Forward (5'→3')**	**Reverse (5'→3')**	**Accession numbers**
TNF-α	GGTGCCTATGTCTCAGCCTCTT	GCCATAGAACTGATGAGAGGGAG	NM_001278601.1
IFN-γ	TCAAGTGGCATAGATGTGGAAGAA	TGGCTCTGCAGGATTTTCATG	NM_008337.4
F4 / 80	CTTTGGCTATGGGCTTCCAGTC	GCAAGGAGGACAGAGTTTATCGTG	NM_001355722.1
COX-2	GCCAGGCTGAACTTCGAAACA	GCTCACGAGGCCACTGATACCTA	NM_011198.4
iNOS	GGAATGGAGACTGTCCCAGCA	GTCATGAGCAAAGGCGCAGA	NM_001313922.1
IL-6	TCCAGTTGCCTTCTTGGGAC	GGTCTGTTGGGAGTGGTATC	NM_001314054.1
IL-1β	GGACCTTCCAGGATGAGGAC	GGTCATCTCGGAGCCTGTAG	NM_008361.4
CCL3	TCTGCAACCAAGTCTTCTCAG	GAAGAGTCCCTCGATGTGGATA	NM_011337.2
GAPDH	TGCTCCTCCCTGTTCCAGA	TACGGCCAAATCCGTTCACA	NM_008084.3

TNF: tumor necrosis factor; IFN: interferon; COX: cyclooxygenase; iNOS: inducible nitric oxide synthase; IL: interleukin; CCL: chemokine (C-C motif) ligand; GAPDH: glyceraldehyde-3-phosphate dehydrogenase.