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Supplementary Materials:Anti-inflammatory Effects of a Polyphenol, Catechin-7,4′-O-digallate, from Woodfordia uniflora by Regulating NF-κB Signaling Pathway in Mouse Macrophages

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General experimental procedure

Optical rotation was measured using Jasco P-2000 polarimeter (Jasco, Easton, MD, USA). Ultraviolet (UV) spectra were obtained using Agilent 8453 UV-visible spectrophotometer (Agilent Technologies, Santa Clara, CA, USA). Nuclear magnetic resonance (NMR) spectra were obtained using Bruker AVANCE III 850 NMR spectrometer with 5 mm TCI CryoProbe, operating at 850 MHz (^{1}H) and 212.5 MHz (^{13}C), with the chemical shift values being represented as parts per million (ppm). Preparative high-performance liquid chromatography (HPLC) was performed using a Waters 1525 Binary HPLC pump with Waters 996 Photodiode Array Detector (Waters Corporation, Milford, CT, USA). Semi-preparative HPLC was performed using the Shimadzu Prominence HPLC System with SPD-20A/20AV Series Prominence HPLC UV-Vis Detectors (Shimadzu, Tokyo, Japan). LC/MS analysis was performed on an Agilent 1200 Series HPLC system, equipped with a diode array detector and 6130 Series ESI mass spectrometer. An analytical Kinetex C_{18} 100 Å column (100 × 2.1 mm, 5 μm; flow rate: 0.3 ml/min; Phenomenex) was used. Furthermore, the Dianion HP-20 system (Mitsubishi Chemical, Tokyo, Japan) along with silica gel 60 (Merck, 230–400 mesh) and RP-C_{18} silica gel (Merck, 40–63 μm) were used for column chromatography, Merck pre-coated Silica gel F254 plates and RP-18 F254s plates (Merck, Darmstadt, Germany) were used for thin-layer chromatography (TLC). The plates were visualized under UV light or by heating after spraying with anisaldehyde-sulfuric acid until the development of spots.

Plant material

Leaves of *W. uniflora* were collected from the valleys of Ain Jarziz, which is approximately 17–18 km east of Salalah, Dhofar (Oman), during June–August 2017. The leaves were dried at room temperature, macerated into a fine powder, and stored at room temperature. The plant was classified by Alan Radcliffe-Smith (Royal Botanic Gardens Kew, England) [1]. A voucher specimen of the material (W-2017) was deposited at the Biology Department, Sultan Qaboos University, Sultanate of Oman.

Reference

1.Miller, A.G.; Morris, M.; Stuart-Smith, S. *Plants of Dhofar, the southern region of Oman: traditional, economic, and medicinal uses*; Office of the Advisor for Conservation of the Environment, Diwan of Royal Court., Sultanate of Oman: 1988.

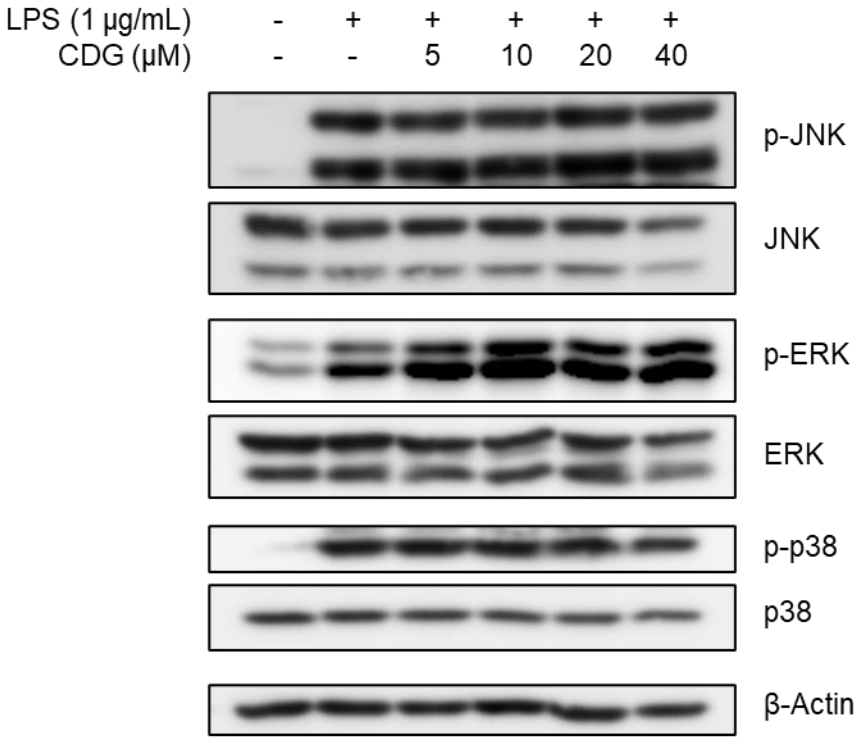

Figure 1. Effect of CDG on the MAPK pathway. The ImKC cells were treated with CDG (5, 10, 20, and 40 μM) for 2 h and stimulated by LPS (1 μg/mL) for 15 min. The cell lysates were used for the analysis of the phosphorylation of MAPK (ERK, JNK, and p38) by western blotting. β-actin was used as the loading control. CDG, catechin-7,4′-O-digallate; MAPK, mitogen-activated protein kinase; LPS, lipopolysaccharide. ERK, extracellular signal-regulated kinase; JNK, c-Jun N-terminal kinase.

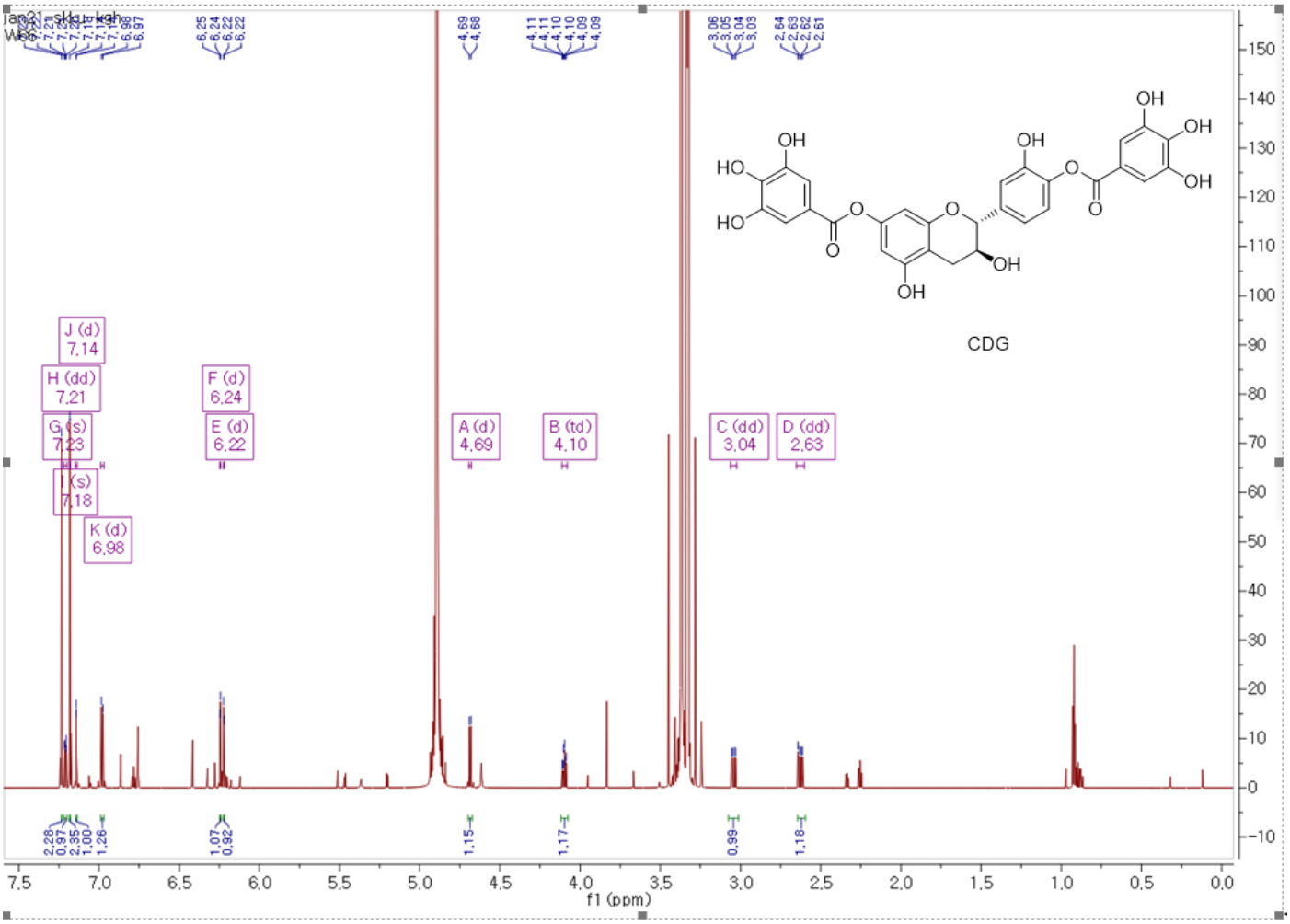

Figure S2. ^{1}H NMR spectrum of catechin-7,4′-*O*-digallate (CDG) in CD_3OD.

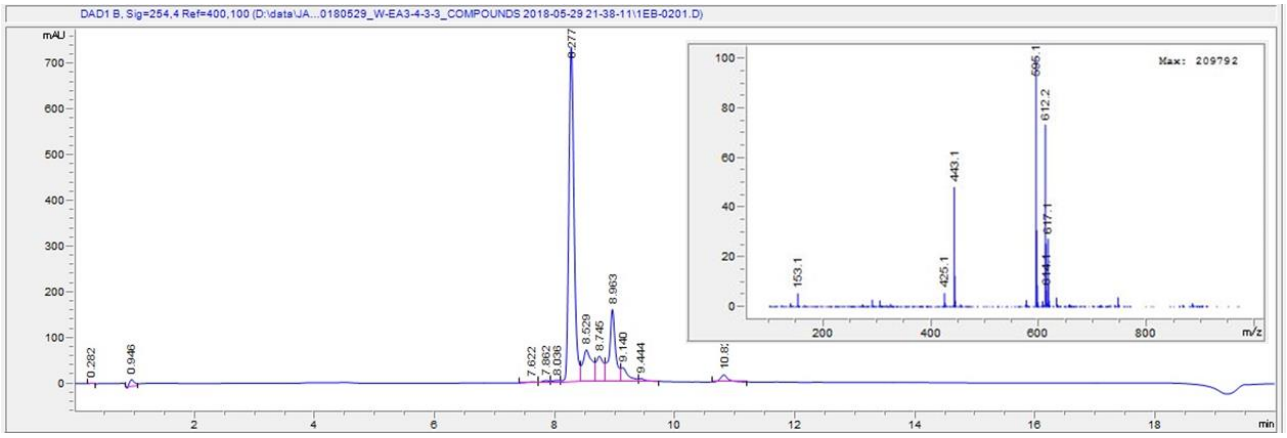

Figure S3. LC/MS analysis of CDG [ESIMS (positive-ion mode) *m*/z: 595.1 $[M + H]^+$ and *m*/z: 617.1 $[M + Na]^+$. Detection wavelength was set at 254 nm; LC/MS analysis was performed on an Agilent 1200 Series HPLC system equipped with a diode array detector and 6130 Series ESI mass spectrometer using an analytical Kinetex C18 100 Å column (100 × 2.1 mm, 5 µm; flow rate: 0.3 mL/min; Phenomenex). The mobile phase was composed of 0.1% (w/v) formic acid in water (solvent A) and methanol (solvent B) in the following gradients; 0 min (A:B 90:10), 10 min (A:B 10:90), 11 min (A:B 0:100), 15 min (A:B 0:100), 16 min (A:B 90:10), and 20 min (A:B 90:10).].

Table S1. List of the primer sequences for qPCR. Primer sequences for inflammatory mediators and housekeeping genes. qPCR, quantitative PCR; iNOS, inducible nitric oxide synthase; IL, interleukin; TNF, tumor necrosis factor; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

Target gene		**Primer Sequence**
iNOS	Sense	5′-TGG CCA AGC TGA ACT-3′
	Anti-Sense	5′-TCA TGA TAA CGT TTC TGG CTC TT-3′
IL-6	Sense	5′-TCT AAT TCA TAT CTT CAA CCA AGA GG -3′
	Anti-Sense	5′-TGG TCC TTA GCC ACT CCT TC-3′
IL-1β	Sense	5′-TTG ACG GAC CCC AAA AGA-3′
	Anti-Sense	5′-GAT GTG CTG CTG CGA GAT T-3′
TNF-α	Sense	5′-CTG TAG CCC ACG TCG TAG C-3′
	Anti-Sense	5′-TTG AGA TCC ATG CCG TTG-3′
GAPDH	Sense	5′-AAC TTT GGC ATT GTG GG-3′
	Anti-Sense	5′-ACA CAT TGG GGG TAG GAA CA-3′
β-actin	Sense	5′-TGT TAC CAA CTG GGA CGA CA-3′
	Anti-Sense	5′-GGG GTG TTG AAG GTC TCA AA-3′