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Short communication

# Phenolic compounds isolated from *Zingiber officinale* roots inhibit cell adhesion

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ABSTRACT

Inhibitors of cell adhesion molecule-mediated cell adhesion might be novel therapeutic agents for the treatment of various inflammatory diseases. In this study, nine phenolic compounds were isolated from the methanol extracts of *Zingiber officinale* roots by bioactivity-guided fractionation. The structures of the compounds were determined by spectroscopic analysis ($^{1}H$, $^{13}C$ NMR and MS), to be 6-gingerol (**1**), 8-gingerol (**2**), 10-gingerol (**3**), 6-shogaol (**4**), 8-shogaol (**5**), 10-shogaol (**6**), dehydro-6-gingerdione (**7**), dehydro-10-gingerdione (**8**) and 6-paradol (**9**). Compounds **3**, **4**, **5** and **7** inhibited direct binding between sICAM-1 and LFA-1 of the THP-1 cells in a dose-dependent manner with $IC_{50}$ values of 57.6, 27.1, 65.4 and 62.0 μM, respectively. Compounds **4** and **7** had an inhibitory effect on direct binding between sVCAM-1 and VLA-4 of THP-1 cells. These results suggest that the phenolic compounds from *Z. officinale* roots are good candidates for therapeutic strategies aimed at inflammation.



## 1. Introduction

Adhesive interactions between circulating monocytes, leukocytes and endothelial cells are the primary event in the initiation of inflammation. This interaction is mediated by adhesion molecules, such as selectins, integrins, Ig-like supergene family of proteins, as intercellular adhesion molecules (ICAMs), and vascular cell adhesion molecules (VCAMs) (Harlan & Winn, 2002; Springer, 1994; Von Andrian & Mackay, 2000). Leukocyte function-associated antigen-1 (LFA-1) is a heterodimeric glycoprotein of the alpha and beta chain (CD11a/CD18b or $\alpha_L\beta_2$), and has been reported in various types of inflammatory cells, including monocytes, leukocytes and macrophages (Hogg et al., 2002; Springer, 1990). It interacts with at least three members of the Ig-like supergene family of proteins, ICAM-1, ICAM-2, and ICAM-3 (Shimaoka & Springer, 2004; Staunton, Marlin, Stratowa, Dustin, & Springer, 1988). In particular, LFA-1/ICAM-1 interactions are considered one of the major pairs of adhesion molecules in the progression of inflammatory diseases, such as rheumatoid arthritis, stroke, psoriasis, allergy and atherosclerosis (Cotran & Mayadas-Norton, 1998; Gottlieb et al., 2002). Therefore, inhibitors of LFA-1/ICAM-1-mediated cell adhesion may be used as novel therapeutic agents for the treatment of inflammatory diseases. Recently, antibodies and small molecule antagonists for the ICAM-1 ligand-binding site or LFA-1 I domain, interfering with LFA-1/ICAM-1 interactions, have been evaluated in many pre-clinical studies. These showed variable efficacy in several animal models of autoimmune disease, collagen-induced arthritis and transplantation (Berlin-Rufenach et al., 1999; Kakimoto et al., 1992; Liu, 2001). In addition, a humanized anti-LFA-1 antibody, such as efalizumab, was approved for the treatment of psoriasis symptoms (Gottlieb et al., 2000). As part of an ongoing search for cell adhesion inhibitors from natural sources, the MeOH extracts of *Zingiber officinale* (Zingiberaceae) were found to have inhibitory activity in a cell adhesion assay. *Z. officinale* is used worldwide as both a spice and medicine (Afzal, Al-Hadidi, Menon, Pesek, & Dhami, 1992; Awang, 1992). It has been used to treat stomach ache, diarrhea, stroke, diabetes, asthma, toothache and arthritis (Tapsell et al., 2006; Wang & Wang, 2005). The roots of this plant were also reported to contain a variety of sesquiterpenes, diarylheptanoids and gingerol-related compounds (gingerols and shogaols) as major constituents. Indeed, several studies have demonstrated that gingerol-related compounds, isolated from *Z. officinale*, have anti-inflammatory, anti-emetic, anti-tumour, anti-oxidation and analgesic properties (Eguchi, Murakami, & Ohigashi, 2005; Lantz et al., 2007; Sharma, Kochupillai, Gupta, Seth, & Gupta, 1997; Surh, 2002). This paper reports procedures for the isolation and structural elucidation of phenolic compounds (**1**–**9**) from the dried roots of *Z. officinale*, as well as their inhibition of the binding between THP-1 cells and cell adhesion molecules.

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## 2. Materials and methods

### 2.1. Analytical equipment

The $^1$H NMR (400, 500 and 600 MHz) and $^{13}$C NMR (100, 125 and 150 MHz) spectra were obtained on JEOL ECS400, JEOL ECX500 and Bruker Biospin Avance 600 spectrometers, with $CDCl_3$ as a solvent. The ESI-MS was determined, using an Agilent 6430 LC/MS/MS and 1100 LC/MS spectrometer. The HPLC system consisted of a Hitachi Model L-2130 pump, L-2400 UV detector and YMC J'sphere ODS H-80 column (4 mm, $\phi$ 20 × 150 mm, YMC Co. Ltd.). Reversed-phase CC was carried out using RP-C18 silica gel (Cosmosil 140 C18-PREP, 140 μm, Nacalai tesque, Inc.). Silica gel CC was conducted using Kieselgel 60 (70–230 and 230–400 mesh, Merck). TLC was conducted using Kieselgel 60 F254 plates (Merck).

### 2.2. Extraction and isolation

The *Z. officinale* roots were purchased at a herbal market in Daejeon, Korea. The authenticity of the plants was confirmed by Prof. K.H. Bae, at the College of Pharmacy of Chungnam National University. A voucher specimen (PBC-022A) was deposited in the Korea Plant Extract Bank, at the Korea Research Institute of Bioscience and Biotechnology. Dried roots of *Z. officinale* (1200 g) were extracted with MeOH (2 l) for 7 days at room temperature. The MeOH extract was evaporated *in vacuo*, yielding a residue (211 g). The residue was suspended in distilled water (1 l) and extracted with EtOAc (1 l). The EtOAc extract was then evaporated *in vacuo*. The residue (124 g) was subjected to silica gel (230–400 mesh, 1 kg, Merck) column chromatography, using a gradient of hexane–EtOAc (100:1, 90:1, 70:1, 50:1, 30:1, 15:1, 5:1, 1:1; each 2 l, v/v) of eluent yielded 19 fractions (F1–19) by TLC profile. Each of the fractions was evaluated for their cell adhesion inhibitory activity. F14 (14.8 g) was subjected to reverse-phase column chromatography (300 g) eluted with MeOH–$H_2O$ (70:30, 80:20, 90:10, 100:0; each 2 l, v/v) yielded 3 sub-fractions (F14-1–3) based on the TLC profile. F14-1 (2.5 g) and F14-3 (1.3 g) were separated successively by semi-preparative HPLC (YMC-Pack ODS-H80, flow rate 4 ml/min), eluted with MeOH–$H_2O$ (80:20, v/v), yielding compounds 1 (948 mg, $t_R$ 28 min) and 3 (764 mg, $t_R$ 35 min), respectively. F14-2 (1.1 g) was subjected to semi-preparative HPLC (MeOH–$H_2O$, 70:30, v/v), yielding compound 2 (613 mg, $t_R$ 54 min). Fractions F8 (9.38 g) and F7 (10.7 g) were subjected to reverse-phase column chromatography (200 g), eluted with MeOH–$H_2O$ (70:30, 80:20, 90:10, 100:0; each 2 l, v/v) yielded three and five sub-fractions (F8-1–3 and F7-1–5). F8-2 (511 mg) and F8-3 (893 mg) were separated successively by semi-preparative HPLC (MeOH–$H_2O$, 80:20 and 75:25, v/v), yielding compounds 4 (141 mg, $t_R$ 8.6 min), 5 (93 mg, $t_R$ 13.9 min) and 9 (113 mg, $t_R$ 11.4 min), respectively. F7-2 (428 mg), F7-3 (304 mg) and F7-5 (272 mg) were subjected to semi-preparative HPLC (MeOH–$H_2O$ = 70:30, 75:25, 90:10, v/v), to yield compounds 6 (63 mg, $t_R$ 15.7 min), 7 (102 mg, $t_R$ 20.4 min) and 8 (78 mg, $t_R$ 31.8 min), respectively.

*6-Gingerol* (**1**): Yellow oil. $C_{17}H_{26}O_4$. ESI-MS *m/z*: 293 $[M–H]^+$. $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ 0.87 (3H, t, *J* = 7.0 Hz, H-10), 1.23–1.48 (8H, m, H-6, H-7, H-8, H-9), 2.47 (1H, dd, *J* = 17.5, 8.6 Hz, H-4), 2.53 (1H, dd, *J* = 17.4, 3.3 Hz, H-5), 2.70 (2H, m, H-2), 2.80 (2H, m, H-1), 3.86 (3H, s, –$OCH_3$), 6.62 (1H, dd, *J* = 8.0, 2.0 Hz, H-6′), 6.65 (1H, d, *J* = 2.0 Hz, H-2′), 6.78 (1H, d, *J* = 8.0 Hz, H-5′). $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ 211.6, 146.5, 144.1, 132.7, 120.8, 114.5, 111.1, 67.8, 56.0, 49.4, 45.5, 36.5, 31.8, 29.4, 25.2, 22.7, 14.1.

*8-Gingerol* (**2**): Brown powder. $C_{19}H_{30}O_4$. EI-MS *m/z*: 321 $[M]^+$. $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ 0.87 (3H, t, *J* = 6.9 Hz, H-12), 1.26–1.49 (12H, m, H-6, H-7, H-8, H-9, H-10, H-11), 2.48 (1H, dd, *J* = 17.5, 9.2 Hz, H-4), 2.56 (1H, dd, *J* = 17.5, 2.9 Hz, H-5), 2.72 (2H, m, H-2), 2.82 (2H, m, H-1), 3.86 (3H, s, –$OCH_3$), 6.65 (1H, dd, *J* = 8.0, 1.9 Hz, H-6′), 6.67 (1H, d, *J* = 1.7 Hz, H-2′), 6.83 (1H, d, *J* = 8.0 Hz, H-5′). $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ 211.6, 146.5, 144.1, 132.7, 120.8, 114.5, 111.1, 67.8, 56.0, 49.4, 45.5, 36.6, 31.9, 29.6, 29.4, 29.3, 25.5, 22.7, 14.2.

*10-Gingerol* (**3**): Colourless powder. $C_{21}H_{34}O_4$. EI-MS *m/z*: 349 $[M–H]^+$. $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ 0.87 (3H, t, *J* = 7 Hz, H-14), 1.25–1.37 (16H, m, H-6, H-7, H-8, H-9, H-10, H-11, H-12, H-13), 2.48 (1H, dd, *J* = 17.5, 8.9 Hz, H-4), 2.56 (1H, dd, *J* = 17.5, 2.9 Hz, H-5), 2.72 (2H, m, H-2), 2.83 (2H, m, H-1), 3.86 (3H, s, –$OCH_3$), 6.65 (1H, dd, *J* = 7.9, 1.9 Hz, H-6′), 6.67 (1H, d, *J* = 2 Hz, H-2′), 6.81 (1H, d, *J* = 8.1 Hz, H-5′). $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ 211.6, 146.5, 144.1, 132.7, 120.8, 114.5, 111.1, 67.8, 56.0, 49.4, 45.5, 36.6, 32.0, 29.7, 29.6, 29.4, 25.5, 22.8, 14.2.

*6-Shogaol* (**4**): Yellow oil. $C_{17}H_{24}O_3$. ESI-MS *m/z*: 274 $[M–H]^+$. $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ 0.87 (3H, t, *J* = 7 Hz, H-10), 1.23–1.45 (6H, m, H-7, H-8, H-9), 2.15–2.19 (2H, m, H-6), 2.79–2.86 (4H, m, H-1, H-2), 3.80 (3H, s, –$OCH_3$), 6.07 (1H, dt, *J* = 16.1, 1.4 Hz, H-4), 6.66 (1H, dd, *J* = 8, 2 Hz, H-6′), 6.69 (1H, d, *J* = 1.7 Hz, H-2′), 6.80 (1H, d, *J* = 5.9 Hz, H-5′), 6.95 (1H, dt, *J* = 15.8, 7.0 Hz, H-5). $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ 200.0, 148.1, 146.6, 144.1, 133.43, 130.5, 121.0, 114.5, 111.3, 56.1, 42.2, 32.6, 31.5, 29.9, 28.0, 22.6, 14.1.

*8-Shogaol* (**5**): Yellow oil. $C_{19}H_{28}O_3$. ESI-MS *m/z*: 304 $[M]^+$. $^1$H NMR (600 MHz, $CDCl_3$): $\delta$ 0.88 (3H, t, *J* = 8.1 Hz, H-12), 1.25–1.45 (10H, m, H-7, H-8, H-9, H-10, H-11), 2.17–2.22 (2H, m, H-6), 2.78–2.88 (4H, m, H-1, H-2), 3.87 (3H, s, –$OCH_3$), 6.10 (1H, dt, *J* = 19.2, 1.8 Hz, H-4), 6.68 (1H, dd, *J* = 9.9, 2.1 Hz, H-6′), 6.71 (1H, d, *J* = 1.8 Hz, H-2′), 6.83 (1H, d, *J* = 9.6 Hz, H-5′), 6.80–6.85 (1H, m, H-5). $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 199.8, 147.9, 146.3, 143.8, 133.2, 130.3, 120.8, 114.3, 111.1, 55.8, 42.0, 32.5, 31.7, 29.8, 29.1, 29.0, 28.1, 22.6, 14.0.

*10-Shogaol* (**6**): Dark yellow oil. $C_{21}H_{32}O_3$. ESI-MS *m/z*: 332 $[M]^+$. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 0.86 (3H, t, *J* = 6.84 Hz, H-14), 1.24–1.43 (14H, m, H-7, H-8, H-9, H-10, H-11,H-12, H-13), 2.16–2.20 (2H, m, H-6), 2.81–2.83 (4H, m, H-1, H-2), 3.85 (3H, s, –$OCH_3$), 6.05 (1H, dt, *J* = 16.1, 1.5 Hz, H-4), 6.68 (1H, dd, *J* = 7.8, 1.9 Hz, H-6′), 6.71 (1H, d, *J* = 1.5 Hz, H-2′), 6.83 (1H, d, *J* = 7.8 Hz, H-5′), 6.78–6.85 (1H, m, H-5). $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 200.0, 148.1, 146.6, 144.1, 133.5, 130.5, 121.0, 114.5, 111.3, 56.1, 42.2, 32.1, 30.1, 29.7, 29.6, 29.5, 29.4, 28.3, 22.9, 14.3.

*Dehydro-6-gingerdione* (**7**): Yellow powder. $C_{17}H_{22}O_4$. ESI-MS *m/z*: 289 $[M]^+$. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 0.91 (3H, t, *J* = 6.84 Hz, H-10), 1.31–1.35 (4H, m, H-8, H-9), 1.58–1.69 (2H, m, H-7), 2.38 (2H, t, *J* = 7.56 Hz, H-6), 3.94 (3H, s, –$OCH_3$), 5.63 (1H, s, H-4), 6.34 (1H, d, *J* = 16.08 Hz, H-1), 6.92 (1H, d, *J* = 8.3 Hz, H-5′), 7.02 (1H, d, *J* = 1.5 Hz, H-4′), 7.09 (1H, dd, *J* = 8.3, 2.0 Hz, H-2′), 7.53 (1H, d, *J* = 15.64 Hz, H-2). $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 200.4, 178.3, 147.9, 147.0, 140.0, 128.0, 122.8, 120.8, 115.0, 109.7, 100.3, 56.2, 40.3, 31.7, 25.5, 22.7, 14.1.

*Dehydro-10-gingerdione* (**8**): Yellow powder. $C_{21}H_{30}O_4$. ESI-MS *m/z*: 330.8 $[M]^+$. $^1$H NMR (400 MHz, $CDCl^3$): $\delta$ 0.86 (3H, t, *J* = 6.8 Hz, H-14), 1.25–1.29 (12H, m, H-8, H-9, H-10, H-11, H-12, H-13), 1.55–1.64 (2H, m, H-7), 2.35 (2H, t, *J* = 7.58 Hz, H-6), 3.92 (3H, s, –$OCH_3$), 5.60 (1H, s, H-4), 6.32 (1H, d, *J* = 15.6 Hz, H-1), 6.90 (1H, d, *J* = 7.8 Hz, H-5′), 7.00 (1H, d, *J* = 2.0 Hz, H-4′), 7.09 (1H, dd, *J* = 8.3, 2.0 Hz, H-2′), 7.51 (1H, d, *J* = 15.64 Hz, H-2). $^{13}$C-NMR (100 MHz, $CDCl_3$): $\delta$ 200.4, 178.2, 147.9, 147.0, 140.0, 128.0, 122.8, 120.8, 115.0, 109.7, 100.4, 56.2, 40.4, 32.1, 29.7, 29.6, 29.5, 25.9, 22.9, 14.3.

*6-Paradol* (**9**): Yellow oil. $C_{17}H_{26}O_3$. ESI-MS *m/z*: 276.9 $[M]^+$. $^1$H NMR (600 MHz, $CDCl_3$): $\delta$ 0.87 (3H, t, *J* = 8.4 Hz, H-10), 1.25–1.30 (8H, m, H-6, H-7, H-8, H-9), 1.53–1.58 (2H, d, *J* = 33 Hz, H-5), 2.37 (2H, t, *J* = 9 Hz, H-4), 2.82 (4H, dt, *J* = 9.2, 9 Hz, H-1, H-2), 3.87 (3H, s, –$OCH_3$), 6.66 (1H, dd, *J* = 9.9, 2.1 Hz, H-6′), 6.69 (1H,