Anti-inflammatory Effects of Chloroform Soluble Fraction from *Perilla frutescens* Britton Leaves Produced by Radiation Breeding in RAW264.7 Cells

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Abstract - The present study aimed to determine the anti-inflammatory effects of each solvent fraction of a mutant *Perilla frutescens* produced by radiation breeding. Following extraction with 80% methanol, *P. frutescens* was fractionated in the order of hexane, chloroform, ethyl acetate, and butanol; the chloroform fraction exhibited less cytotoxicity, the greatest inhibitory effect on the production of nitric oxide (NO), and the highest rate of inhibition on the generation of tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), monocyte chemoattractant protein-1 (MCP-1), and interferon-β (IFN-β). The chloroform fraction also suppressed the mRNA and protein levels of inducible nitric oxide synthase (iNOS) and reduced the activation of nuclear factor-κB (NF-κB) in lipopolysaccharide (LPS)-stimulated RAW264.7 cells. Finally, the presence of corosolic acid in the chloroform fraction was identified. Taken together, the present findings indicate that the chloroform fraction obtained from mutant *P. frutescens* inhibited NO production in LPS-stimulated RAW264.7 cells via the suppression of iNOS expression and the inactivation of NF-κB.

Key words : *Perilla frutescens* Britton, Anti-inflammation, Nitric oxide, Inducible nitric oxide synthase, Nuclear factor-κB

INTRODUCTION

Perilla frutescens Britton is an annual herbaceous plant of the Lamiaceae family that naturally grows naturally in East Asia (Lee 1982). In traditional medicinal practices, *P. frutescens* is used to treat a variety of illnesses including cough, phlegm, back pain, and diabetes (Han *et al*. 1994; Kim *et al*. 2007). The ingredients of *P. frutescens* include rosmarinic acid, triterpenoids, luteolin, apigenin, and lignans (Okuda *et al*. 1986; Woo *et al*. 2014). Previous studies have reported that the ethanol extract of *P. frutescens* exerts anti-inflammatory effects (Kim *et al*. 2007; Lee *et al*. 2012), the water and ethanol extracts have antioxidant effects (Cho *et al*. 2011), and its methanol extract may exert a preventative action against Alzheimer's disease (Choi *et al*. 2004). The antioxidant effects of *P. frutescens* appear to be dependent on the inhibitory activity of xanthine oxidase and the radical scavenging activity of 2,2-diphenyl-1-picrylhydrazyl (DPPH; Han *et al*. 2004). Isoegomaketone (IK), which is a fragrant ingredient of *P. frutescens* that has a distinctive aroma and is biosynthesized from egomaketone, has been

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isolated, identified (Madoka *et al*. 2004; Park *et al*. 2009), and shown to exert anti-inflammatory effects through the nuclear factor-κB (NF-κB) pathway and inhibition of the JAK-STAT-1 pathway (Jin *et al*. 2010). Additionally, IK produces anti-obesity effects by inhibiting the expression of adipogenetic genes related to the differentiation, synthesis, and fat accumulation of preadipocyte 3T3-L1 cells (So *et al*. 2015).

The bodily inflammatory response is a defense mechanism against external stimuli that occurs in response to tissue stress, damage, and infection (Cho *et al*. 2009). During this response, macrophages produce inflammatory mediators such as nitric oxide (NO), prostaglandin E2 (PGE2), tumor necrosis factor-α (TNF-α), and interleukin-6 (IL-6; Coussens *et al*. 2002). However, persistent inflammatory responses can result in arthritis, diabetes, arteriosclerosis, and cancer by inducing tissue damage (Libby 2006). Macrophages are activated by oxidative stress, cytokines, and lipopolysaccharides (LPS), which are components of the cell wall in Gram-negative bacteria, act as an endotoxins in the body, and induce the production of cytokines and NO (Stuehr *et al*. 1991). NO is produced from L-arginine by the catalytic reaction of NO synthases (NOSs). Unlike other NOSs such as endothelial NOS and neuronal NOS, inducible nitric oxide synthase (iNOS) does not produce NO under normal conditions. NO generated over a short time period in response to LPS or inflammatory cytokines possesses antioxidant, anti-proliferative and immune effects, but when this factor is overexpressed for a long period of time, it may exhibit toxicity and cause damage and mutations in DNA (Cho *et al*. 2014).

Thus, the present study aimed to investigate the anti-inflammatory effects of various solvent fractions from a mutant *P. frutescens* Britton produced by radiation breeding. Additionally, the most efficacious fraction was used to investigate the mechanisms underlying the anti-inflammatory activities; the ingredients were isolated and structural analyses were performed to determine the compounds that exhibited the anti-inflammatory effects.

METERIALS AND METHODS

Reagents

Dulbeccos's modified eagle medium (DMEM) and fetal bovine serum (FBS) were purchased from Hyclone (Logan, UT, USA). Griess reagent, LPS, NP40 cell lysis buffer, protease inhibitor cocktail, dimethyl sulfoxide (DMSO), and the corosolic acid analytical standard were purchased from Sigma-Aldrich (St. Louis, MO, USA). The EZ-Cytox cell viability assay kit was purchased from DAEIL Lab Service (Seoul, Korea) and TNF-α, IL-6, and monocyte chemoattractant protein-1 (MCP-1) enzyme linked immunosorbent assay (ELISA) kits were purchased from R&D Systems (Minneapolis, MN, USA). The interferon-β (IFN-β) ELISA kit was purchased from Pestak Bio Lab (Piscataway, NJ, USA) and the rabbit polyclonal antibody for iNOS was purchased from Cell Signaling Technology (Danvers, MA, USA). The rabbit polyclonal antibody against β-tubulin was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA) and an RNeasy kit was obtained from QIAGEN (Valencia, CA, USA). An advantage RT-for-PCR kit was purchased from Clontech Laboratories (Mountain View, CA, USA) and the SYBR premix (Takara Bio, Otsu, Japan), Opti-MEMI medium, goat anti-rabbit IgG HRP-conjugated antibody, and Lipofectiamine 2000 were purchased from Invitrogen (Carlsbad, CA, USA).

General experimental procedures

For the ingredient analysis, chromatography was performed using an open column and Silica gel on Kieselgel 60 plates (70~230 mesh, ASTM; Merck, Kenilworth, NJ, USA) and the chromatographic separations were monitored by TLC plates pre-coated with Kieselgel 60 F254 (Art.552; Merck). All analytical and HPLC grade solvents were obtained from Thermo Fisher Scientific (Waltham, MA, USA) and 1H, 13C and nuclear magnetic resonance spectroscopy (NMR) spectra were analyzed with a JNM-ECA500 (JEOL Co., Tokyo, Japan).

Corosolic acid

White powder; 1H-NMR (pyridine-*d*5, 500 MHz): δ 5.40 (1H, brs, H-12), 4.03 (1H, ddd, J = 11.0, 9.5, 4.0 Hz, H-2), 3.33 (1H, d, J = 9.0 Hz, H-3), 2.57 (1H, d, J = 10.5 Hz, H-18), 2.31 (1H, ddd, J = 13.5, 13.0, 4.5 Hz, H-16), 2.13 (1H, ddd, J = 13.0, 12.5, 3.5 Hz, H-1), 1.21 (3H, s, Me-23), 1.15 (3H, s, Me-27), 1.02 (3H, s, Me-26), 0.98 (3H, s, Me-24), 0.92 (3H, s, Me-25), 0.91 (3H, d, J = 6.0 Hz, Me-30), and 0.85 (3H, d, J = 6.5 Hz, Me-29).

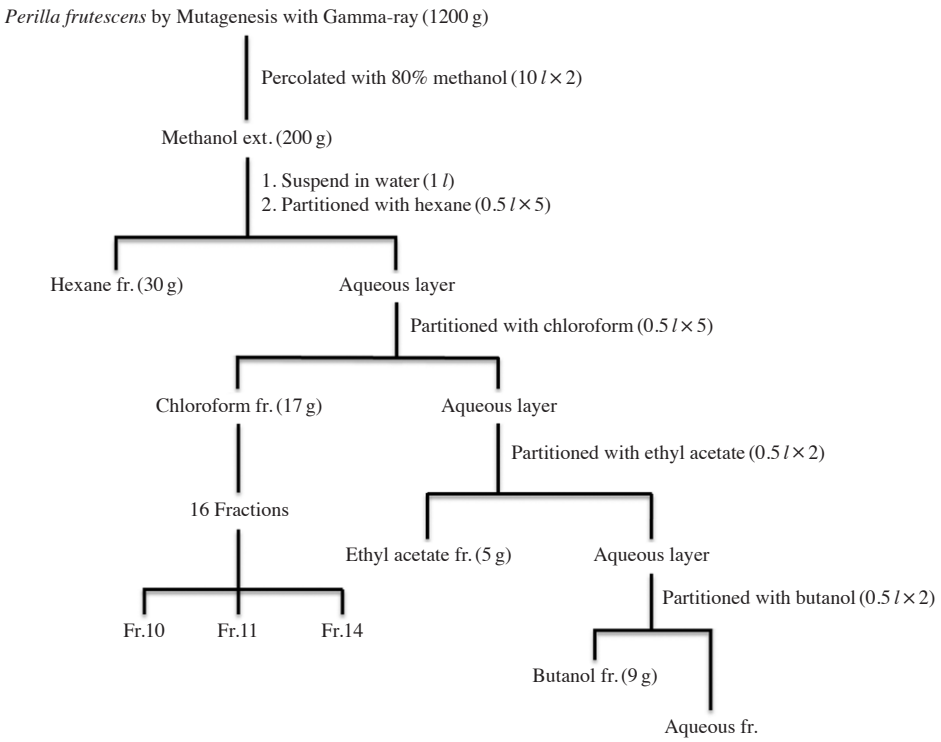

Fig. 1. Extraction and solvent partition of *Perilla frutescens* Britton leaves.

HPLC analysis

The HPLC analysis was performed using an Agilent Eclipse XDB-C18 column (4.6 × 250 mm, 5 μm; Agilent Technologies, Santa Clara, CA, USA) and the mobile phase carried out using water (A) and acetonitrile (B) phases. The gradient elution system conditions were as follows: 0∼5 min, 50% B; 50 min, 95% conditions. The injection volume was 10 μl, the flow rate was 1 ml min^{-1}, the column temperature was maintained at 25°C, and the UV detection wavelength was 220 nm.

Plant material and extract preparation

The mutant *P. frutescens* was provided by the Korea Atomic Energy Research Institute (Daejeon, Korea). The *P. frutescens* leaves were irradiated with a 200 Gy gamma ray for mutagenesis, dried, and then ground to powder (1.2 kg). The powder was extracted with 80% methanol (10 *l*) at room temperature for 24 h and filtered through filter paper. Following extraction, the solution was fractionated in the order of hexane, chloroform, ethyl acetate, and butanol; the systematic preparation scheme is shown in Fig. 1.

Cell culture

RAW264.7 cells were cultured in DMEM supplemented with 10% FBS, streptomycin (100 μg ml^{-1}), and penicillin (100 U ml^{-1}) and then incubated at 37°C in an atmosphere with 5% CO_2.

Cell viability

Cell viability was assessed using an EZ-Cytox cell viability kit (DAEIL). The cells were cultured in a 96-well culture plate at a density of 2×10^5 cells ml^{-1} for 24 h and then treated with each fraction for an additional 24 h. Following treatment, 10 μl of the kit solution was added to each well and the solution was incubated for 4 h at 37°C in an atmosphere with 5% CO_2. Cell viability was measured at 480 nm using a microplate reader (Benchmark Plus; Bio-Rad, Hercules, CA, USA) and a reference wavelength of 640 nm.

NO assay

The cells were seeded in a 96-well plate, pre-incubated with each solvent fraction for 2 h, and then incubated with LPS (1 μg ml^{-1}) for 18 h. Next, 100 μl of culture supernatant was mixed with an equal volume of Griess reagent and

incubated at room temperature for 15 min. The absorbance at 570 nm was measured in a microplate reader with a reference wavelength of 650 nm. The nitrite level in the cellular media was measured using the Griess method (Park *et al*. 2009).

Total protein extracts

The cells were cultured in a 100 mm culture plate at a density of 2×10^5 cells ml^{-1} for 24 h and pre-incubated with each solvent fraction for an additional 2 h. Next, the cells were incubated with LPS (1 μg ml^{-1}) for 18 h and washed once with phosphate buffered saline (PBS), harvested by pipetting, and lysed on ice for 30 min in an NP40 cell lysis buffer containing a protease inhibitor cocktail and phenylmethylsulfonyl fluoride. Next, the cells were centrifuged at 15,000 rpm for 15 min at 4°C. The supernatants were used as total protein extracts and the total protein contents were determined by the Bio-Rad protein assay using bovine serum albumin (BSA) as the standard.

Western blot analyses

Equal amounts of the protein samples (50 μg) were separated on 10% sodium dodecyl sulfate (SDS) polyacrylamide gels and transferred to nitrocellulose membranes. The membranes were blocked with 5% skim-milk in Tris-buffered saline with Tween 20 (TBS-T; 10 mM Tris-HCl, pH 7.4, 150 mM NaCl, and 0.1% Tween 20) for 1 h and then incubated with the iNOS and β-tubulin primary antibodies at 4°C overnight; the rabbit polyclonal antibodies against iNOS (1:1,000), and β-tubulin were diluted (1:200) in blocking buffer. The membranes were washed three times with TBS-T for 15 min and then incubated with anti-rabbit IgG HRP-conjugated secondary antibody diluted in blocking buffer (1:5,000) for 2 h at room temperature. Next, the membranes were washed and the blotted proteins were detected using an enhanced chemiluminescence detection system (Amersham Biosciences, Little Chalfont, UK).

Extraction of total RNA and real-time polymerase chain reaction (RT-PCR)

The cells were cultured in a 6-well culture plate at a density of 2×10^5 cells ml^{-1} for 24 h, pre-incubated with various concentrations of the chloroform fraction for 2 h, and then LPS (1 μg ml^{-1}) was added for 18 h. The total RNA from the cells was isolated using an RNeasy kit (QIAGEN) according to the manufacturer's instructions. A chromo real-time PCR detection system (Bio-Rad) and iTaq-SYBR green supermix (Bio-Rad) were used for the RT-PCR amplification of iNOS and β-actin under the following conditions: 50 cycles of 94°C for 20 s, 60°C for 20 s, and 72°C for 30 s. All reactions were repeated independently at least three times to ensure the reproducibility of the results. The quantitative iNOS and β-actin primers (Bioneer Corp., Chungwon, Korea) were as follows: iNOS forward primer, 5′-TCCTACACCA CACCAAACTGTGTGC-3′, and reverse primer, 5′-CTCC AATCTCTGCCTAT CCGTCTC-3′, β-actin forward primer, 5′-TGAGAGGGAAATCGTGCGTGAC-3′, and reverse primer, 5′-GCTCGTTGCCAATAGTGATGACC-3′. The specificity of the amplified PCR products was assessed with a melting curve analysis and the relative expressions of the target genes compared to the β-actin gene were evaluated using the comparative C_T threshold method and the Bio-Rad software tool Genex-Gene Expression Macro.

ELISA procedure

The cells were cultured in a 6-well culture plate at a density of 2×10^5 cells ml^{-1} for 24 h and then pre-incubated with each solvent fraction for an additional 2 h. Next, the cells were further incubated with LPS (1 μg ml^{-1}) for 4 h (IL-6 and TNF-α), and 18 h (MCP-1 and IFN-β). The cytokine levels were measured using an ELISA kit according to manufacturer's protocols.

Luciferase assay

The cells were cultured in a 6-well culture plate at a density of 2×10^5 cells ml^{-1} for 24 h and the transfection of the vectors was performed using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. The pNF-κB luciferase vector (5 μg) and pRL-TK control reporter vector (1 μg) were transfected using the vector per well. Following the transfection, the chloroform fraction was added at various concentrations for 2 h, and then the cells were treated with LPS (1 μg ml^{-1}) for 24 h. Luciferase activity was measured using the dual-luciferase reporter assay system (Promega, Madison, WI, USA).

Statistical analysis

All data are presented as means ± standard deviation (SD).

The differences between the means of the treated and untreated groups were determined with Student's *t* tests implemented in the Excel program (Microsoft Corp., Redmond, WA, USA); p values < 0.05 were considered to indicate statistical significance.

RESULTS

Effects of various solvent fractions on cell viability and NO production in LPS-stimulated RAW264.7 cells

The cytotoxicity of each solvent fraction was determined using an EZ-Cytox cell viability kit. Following treatment with each fraction at a concentration of 50 μg ml^{-1}, cell viability was greater than 90%; therefore, the experiments were performed at non-cytotoxic concentrations of 50 μg ml^{-1} or less. The NO production was measured using the Griess reagent after treatment with each fraction (50 μg ml^{-1}). NO production largely increased in the LPS-treated group but decreased in all fraction-treated groups, except for the butanol fraction-treated group. In particular, the chloroform fraction inhibited NO production at a rate of 95% (Fig. 2).

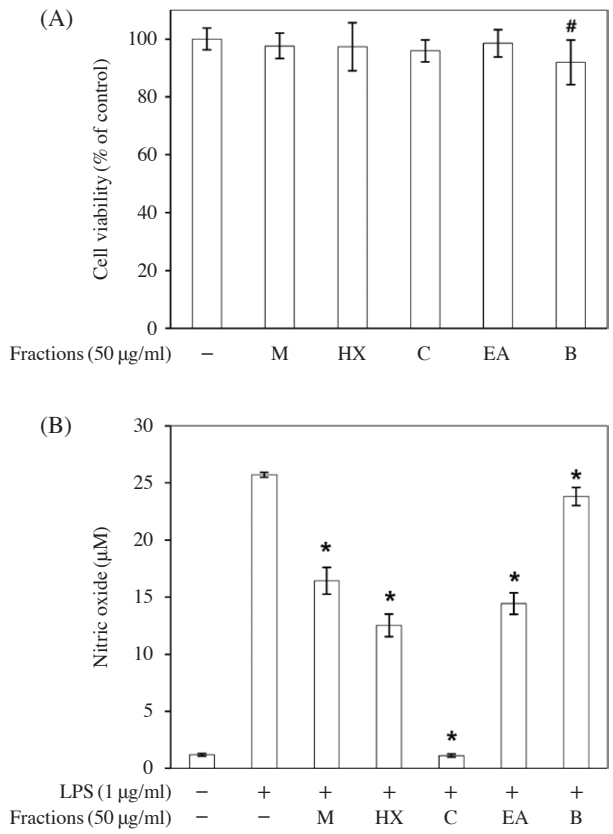

Fig. 2. Effects of various solvent fractions on cell viability and nitric oxide (NO) production in lipopolysaccharide (LPS)-stimulated RAW264.7 cells. (A) Cell viability was evaluated using EZ-Cytox cell viability assay and the relative absorbance values were compared. (B) Cells were treated with the solvent fractions (50 μg ml^{-1}) for 2 h and then incubated in LPS (1 μg ml^{-1}) for 18 h. NO was measured with the culture media using the Griess Reagent. Each value represents the mean ± SD. (n = 6). #$p < 0.05$ vs. control, *$p < 0.05$ vs. LPS alone-treated group. M: methanol, HX: hexane, C: chloroform, EA: ethyl acetate, B: butanol.

Inhibitory effects of solvent fractions on the production of inflammatory cytokines in LPS-stimulated RAW264.7 cells

In the present study, the production level of IL-6, TNF-α, MCP-1, and IFN-β were measured using ELISA kits after treatment with each fraction (50 μg ml^{-1}). When RAW264.7 cells were stimulated with LPS, the levels of IL-6 were increased, but the chloroform fraction inhibited this increase by 85% (Fig. 3A). The chloroform fraction inhibited TNF-α by 65%, which was the highest level of inhibition among the various fractions (Fig. 3B). Other than the butanol fraction, all fractions decreased MCP-1 production and the chloroform fraction had the highest rate of inhibition of MCP-1 (Fig. 3C). The hexane and chloroform fractions had inhibition rates of 61%, and 68%, respectively, for IFN-β (Fig. 3D).

Effects of the chloroform fraction on cell viability and NO production in LPS-stimulated RAW264.7 cells

Cell viability was measured after administration of the chloroform fraction at various concentrations (6.25, 12.5, 25, and 50 μg ml^{-1}); no cytotoxicity was observed at any concentration (Fig. 4A). On the other hand, NO production decreased in a concentration-dependent manner following treatment with the chloroform fraction (Fig. 4B); the IC_{50} was 12.58 μg ml^{-1}.

Effects of the chloroform fractions on iNOS expression levels in LPS-stimulated RAW264.7 cells

The mRNA and protein levels of iNOS increased in the LPS-treated group but iNOS expression was suppressed in a concentration-dependent manner following treatment with

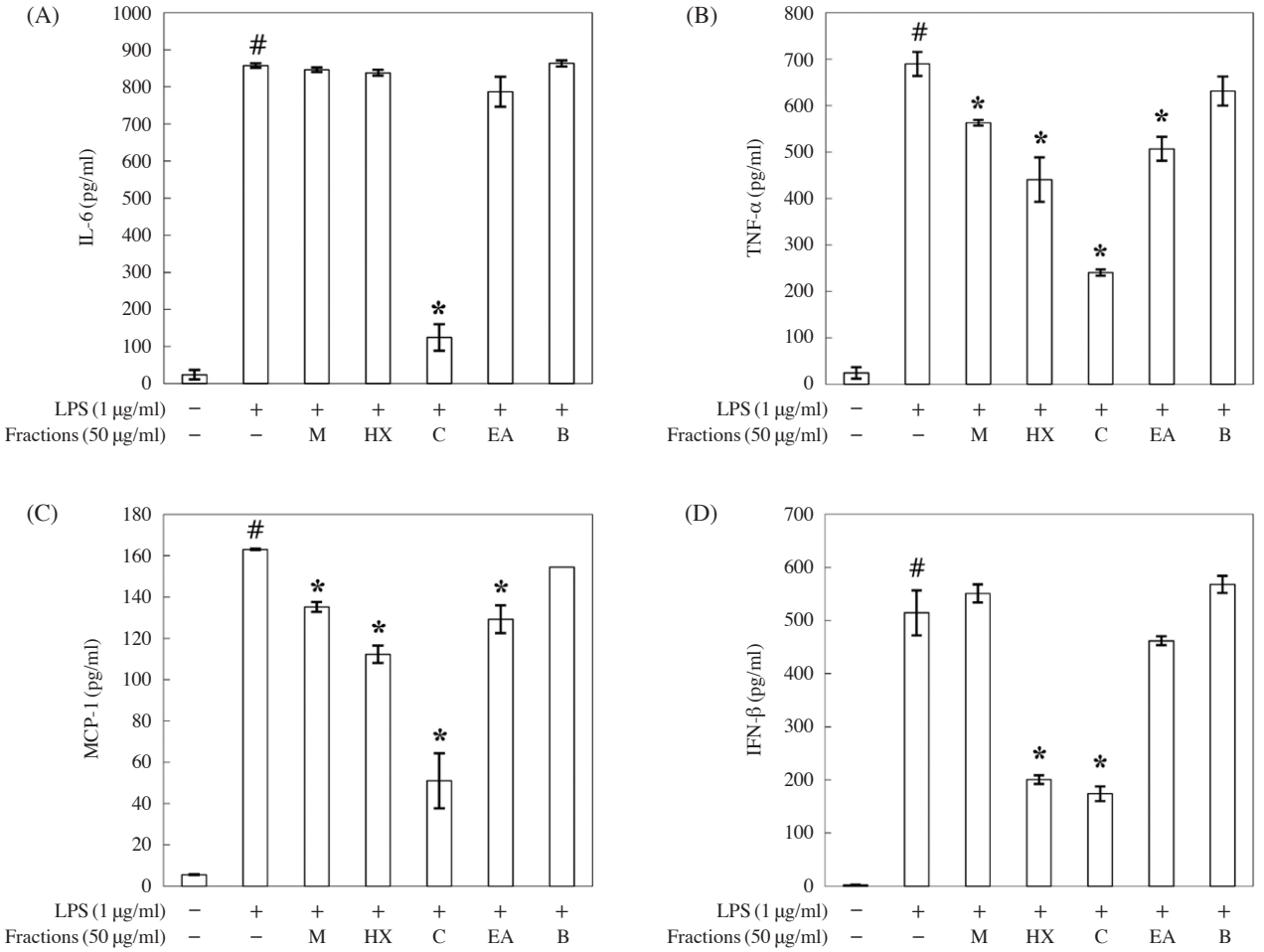

Fig. 3. Inhibitory effects of the solvent fractions on interleukin (IL-6), tumor necrosis factor-α (TNF-α), monocyte chemoattractant protein-1 (MCP-1), and interferon-β (IFN-β) production levels in LPS stimulated RAW264.7 cells. The cells were pre-incubated with various solvent fractions (50 μg ml^{-1}) for 2 h and then incubated with LPS (1 μg ml^{-1}) for 4 h (IL-6 and TNF-α) or 18 h (MCP-1 and IFN-β). Cytokines were measured with enzyme-linked immunosorbent assay (ELISA) assays. Each value represents the mean ± SD. (n = 3). $^{\#}p < 0.05$ vs. control, $^{*}p < 0.05$ vs. LPS alone-treated group.

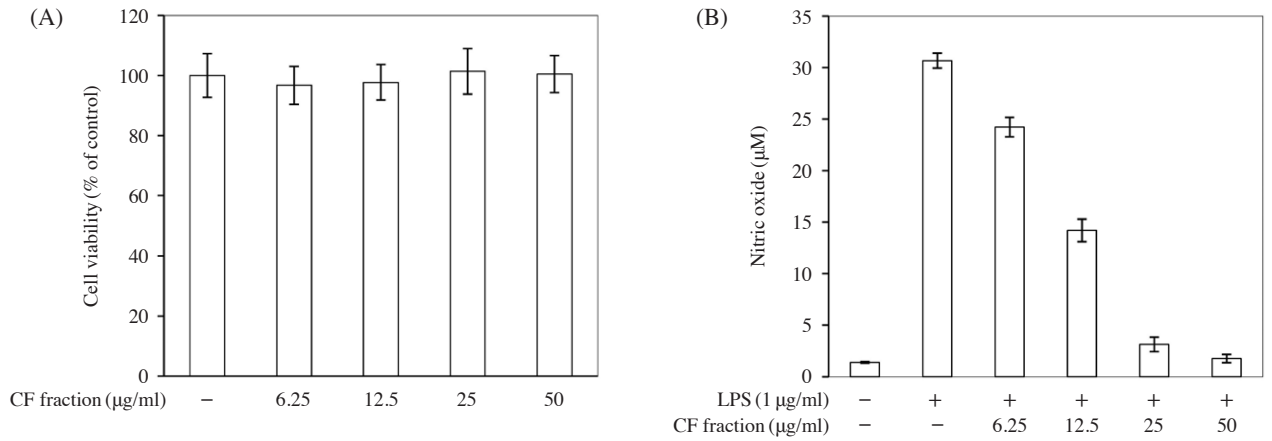

Fig. 4. Effects of the chloroform fractions on cell viability and NO production in LPS-stimulated RAW264.7 cells. (A) Cell viability was evaluated using EZ-Cytox cell viability assay and relative absorbance values were compared. (B) Cells were treated with different concentrations of the chloroform fraction (6.25, 12.5, 25, and 50 μg ml^{-1}) for 2 h and then incubated in LPS (1 μg ml^{-1}) for 18 h. NO was measured with the culture media using Griess reagent. Each value represents the mean ± SD. (n = 6).

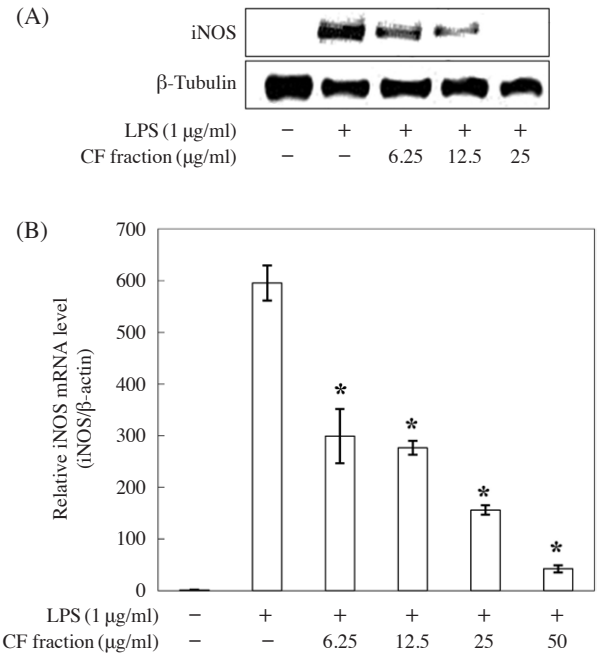

Fig. 5. Effects of the chloroform fraction on inducible nitric oxide synthase (iNOS) expression levels in LPS-stimulated RAW264.7 cells. (A) The expressions of the iNOS and β-tubulin proteins were analyzed with Western blot analyses. Cells were treated with different concentrations of the chloroform fraction (6.25, 12.5, and 25 μg ml^{-1}) for 2 h and then incubated with LPS (1 μg ml^{-1}) for 18 h. (B) iNOS mRNA levels were determined with an real-time polymerase chain reaction (RT-PCR) assay. The mRNA expression of iNOS was determined in the cells stimulated by LPS (1 μg ml^{-1}) in the presence of different concentration of the chloroform fraction (6.25, 12.5, and 25 μg ml^{-1}) for 24 h. Each value represents the mean ± SD. (n = 3). *$p < 0.05$ vs. LPS alone-treated group.

the various chloroform factions (Fig. 5). These findings indicate that NO production was decreased by the inhibition of iNOS expression. Therefore, it appears that the anti-inflammatory effects of *P. frutescens* are the greatest in the chloroform fraction.

Effects of the chloroform fractions on NF-κB activation in LPS-stimulated RAW264.7 cells

To determine whether NF-κB activation was influenced by treatment with the chloroform fraction, its activation levels were measured using a luciferase assay. Compared with the non-LPS-treated group, NF-κB activation was suppressed by approximately six-fold in the LPS-treated group. Additionally, NF-κB was inhibited in a concentration-dependent manner when treated with the various concentrations of the chloroform faction (Fig. 6).

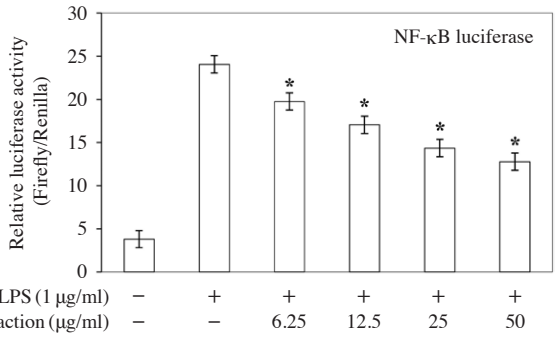

Fig. 6. Effects of the chloroform fraction on nuclear factor-κB (NF-κB) activation in LPS-stimulated RAW264.7 cells. The cells were transfected with 5 μg of pNF-κB luciferase and 1 μg of pRL-TK control reporter vector. The cells were treated with the chloroform fraction prior to treatment with LPS (1 μg ml^{-1}) and then incubated for 24 h. Each value represents the mean ± SD. (n = 3). *$p < 0.05$ vs. LPS alone-treated group.

Ingredient isolation and structural analyses of the active components

The chloroform fraction exhibited significant anti-inflammatory effects relative to the other solvent fractions. This fraction was divided into 16 sub-fractions using Silica gel column chromatography with a chloroform:methanol ratio of 100 : 1. Sub-fraction 14 had inhibitory effects on NO production without cytotoxicity and, thus, was structurally analyzed using NMR. The structure of this isolated single ingredient was identified by comparing the present findings with the NMR data of previous papers (Woo *et al*. 2014); the ingredient contained in sub-fraction 14 determined to be corosolic acid (Fig. 7A). This result was confirmed by high-performance liquid chromatography (HPLC) using a corosolic acid analytical standard (Fig. 7B, C).

DISCUSSION

The anti-inflammatory effects of *P. frutescens* have been demonstrated using various extraction methods (Choi *et al*. 2004; Kim *et al*. 2007; Cho *et al*. 2011; Lee *et al*. 2012). In other study, after extraction with 80% ethanol, *P. frutescens* was fractionated in the order of hexane, ethyl acetate, and butanol. Among these fractions, ethyl acetate fraction has the highest reported inhibitory effects on NO and PGE2 production (Kim *et al*. 2006). In contrast, the present study found that the ethyl acetate fraction had a lower inhibitory effect on NO production than the chloroform fraction, which

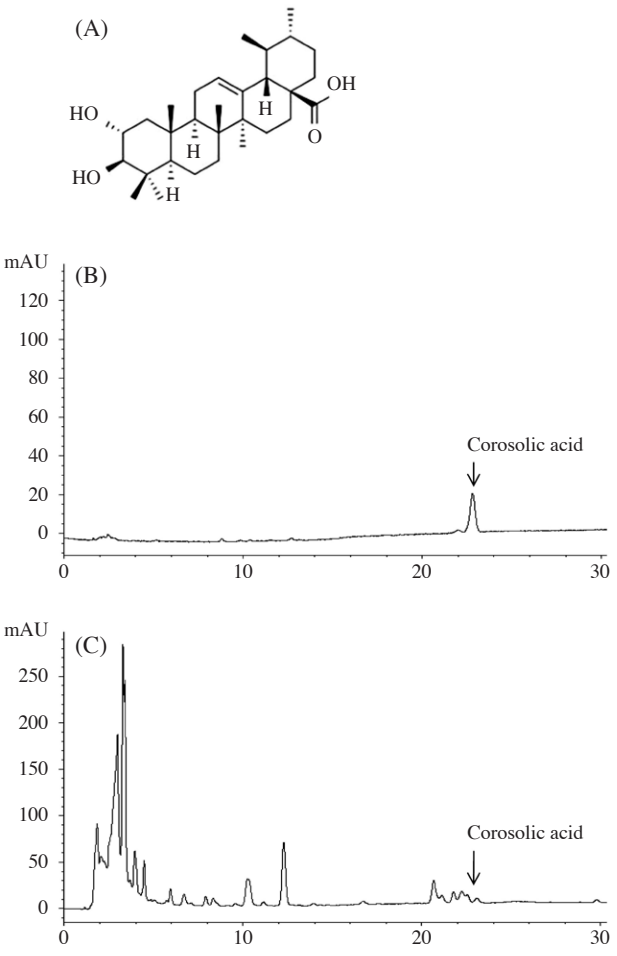

Fig. 7. Structure of corosolic acid and the high-performance liquid chromatography (HPLC) chromatogram of corosolic acid at 210 nm. (A) Molecular structure of the corosolic acid. (B) HPLC chromatogram of the corosolic acid standard. (C) Chloroform fraction of *P. frutescens*.

may indicate that the chloroform fraction has a greater anti-inflammatory effect than the ethyl acetate fraction. Furthermore, in a previous study that employed the same extraction method as the present study, the chloroform fraction of *P. frutescens* showed anti-carcinogenic effects (Han *et al*. 1994). Thus, the chloroform fraction of *P. frutescens* could be used for anti-cancer research as well as anti-inflammatory research.

Once activated by LPS, macrophages secrete inflammatory cytokines to mediate the inflammatory response. IL-6 is not only involved in inflammation and infection responses, but also in the regulation of metabolic, regenerative, and neural processes (Kim *et al*. 2004). TNF-α is a cytotoxic molecule that induces tumor necrosis and its production is enhanced at site of inflammation (Park *et al*. 2012). MCP-1 plays an important role in the initiation of the inflammatory response and is secreted by a number of cells related to inflammation including fibroblasts, endothelial cells, vascular smooth muscle cells, mononuclear cells, and T cells (Melarejo *et al*. 2009). IFN-β is a lymphokine produced by T cells that regulates antiviral activity, macrophage activation, and apoptosis (Boehm *et al*. 1997). IFN-β is also associated with the expression of iNOS through the JAK/STAT-1 pathways (Jin *et al*. 2010).

In LPS-stimulated RAW264.7 cells, NO is produced via iNOS expression and the activation of NF-κB (Kwai *et al*. 2006). In the present study, RT-PCR and Western blot analyses were carried out to determine whether the inhibition of NO production by the chloroform fraction in LPS-stimulated RAW264.7 cells was due to decrease in the expression of iNOS. The chloroform fraction induces the inhibition of NO production by suppressing iNOS expression in murine microbial BV-2 cells (Kim *et al*. 2007). NF-κB is an inflammatory transcription factor that regulates the expression of genes involved in the mediation of the inflammatory response, apoptosis, and cell proliferation through the TLR4-MyD88 signal transduction pathways (Kwai *et al*. 2006).

Corosolic acid is a compound that is widely known as a triterpenoid in the leaves of the banabá plant (*Lagerstroemia speciosa* L.; Jayakuma *et al*. 2014). More specifically, this plant is called the "insulin plant" due to its excellent ability to lower blood glucose levels; it has also been reported to have anti-diabetic (Toshihiro *et al*. 2006), anti-carcinogenic (Yangeng *et al*. 2009), and anti-inflammatory (Hong *et al*. 2012) effects. Corosolic acid inhibited the activation of NF-κB during the LPS-stimulated inflammatory response, which suggests that it was responsible for the inhibition of NO activity induced by the chloroform fraction of radiation-bred *P. frutescens*. The content of corosolic acid from chloroform-soluble fraction was 0.3%. Even if the content of that was very small, that had highest anti-inflammatory activity in chloroform-soluble fraction. In the present study, the corosolic acid isolated from the chloroform fraction had a similar inhibitory effect on NO production to that observed in other studies (Hong *et al*. 2012) and many studies have demonstrated the anti-inflammatory properties of *P. frutescens* using various extraction methods (Kim *et al*. 2007; Lee *et al*. 2012). However, no studies have assessed the anti-inflammatory effects of a single ingredient isolated from the chloroform fraction.

CONCLUSION

The present study demonstrated that the chloroform fraction from the radiation-bred mutant *P. frutescens* had strong anti-inflammatory effects. The present findings may serve as a basis for future studies investigating the anti-inflammatory and anti-carcinogenic effects of *P. frutescens*.

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