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Anti-inflammatory and cytotoxic effects of methanol, ethanol, and water extracts of *Angelicae Dahuricae Radix*

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Abstract: *Angelicae Dahuricae Radix* has been used for the treatment of headaches, rhinitis, and colds in traditional medicine. Methanol, ethanol, and water extracts of *Angelicae Dahuricae Radix* were collected. A statistically significant reduction in the cellular viability of the mouse leukemic monocyte macrophage cell line was noted after treatment with water extracts of *Angelicae Dahuricae Radix*. Stimulation with lipopolysaccharides (LPS) for 24 h led to a robust increase in nitric oxide production, but *Angelicae Dahuricae Radix* at 400 µg/mL concentration significantly suppressed nitric oxide produced by the LPS-stimulated RAW 264.7 cells in 70% ethanol, absolute ethanol, 70% methanol, absolute methanol, and boiling water groups ($P < 0.05$). Pretreatment with absolute ethanol extract of *Angelicae Dahuricae Radix* suppressed the LPS-stimulated inducible nitric oxide synthase, interleukin-1 β , and cyclooxygenase-2 expression. *Angelicae Dahuricae Radix* showed significant cytotoxic effects on the human adenocarcinoma cell line and keratin-forming cell line. (J Oral Sci 58, 125-131, 2016)

Keywords: anti-inflammatory agents; antineoplastic agents; herbal medicine; plant roots.

Introduction

Angelica dahurica is a wild species of *Angelica* native to Siberia, Russian Far East, Mongolia, Northeastern China, Japan, Korea, and Taiwan. It is a perennial plant also commonly known as Chinese Angelica, Wild Angelica, or Bai Zhi in Chinese (1). *Angelicae Dahuricae Radix* is the dried root of *Angelica dahurica* (Fisch. ex Hoffm.) Benth. et Hook. f. and *Angelicae dahurica* (Fisch. ex Hoffm.) Benth. et Hook. f. var. *formosana* (Boiss.) Shan et Yuan (Fam. Umbelliferae). In addition to being used for the treatment of headaches, rhinitis, and colds in traditional medicine (2), *Angelicae Dahuricae Radix* also serves as a pain killer, anti-inflammatory agent, laxative, and sedative, as well as a treatment for toothache and periodontitis (3-5).

The anti-cancer properties of *Angelicae Dahuricae Radix* have been previously reported (6-7). Methanol extracts of *Angelicae Dahuricae Radix* exhibited significant cytotoxicity against cultured human tumor cell lines *in vitro*. Moreover, the hexane soluble part of the extract significantly inhibited the proliferation of cultured human tumor cells, while the remaining water soluble part exhibited poor inhibition (6). The anti-inflammatory effects were tested using methanol extracts of *Angelicae Dahuricae Radix* on acetic acid-induced vascular permeability, carrageenan-induced paw edema, and myeloperoxidase activity (8). Inducible nitric oxide

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synthase (iNOS) expression and nitric oxide production were significantly suppressed by the methanol extract of *Angelicae Dahuricae Radix* in a dose-dependent manner (8).

The aim of this study was to determine the dose-dependent, anti-inflammatory, and cytotoxic effects using methanol, ethanol, and water extracts of *Angelicae Dahuricae Radix*. To the best of our knowledge, this study is the first to compare the effects of methanol, ethanol, and water extracts of *Angelicae Dahuricae Radix* on the mouse leukemic monocyte macrophage cell line (RAW 264.7), human prostate cancer cell line (PC-3 cells), and human adenocarcinoma cell line (A549 cells).

Materials and Methods

Chemicals and reagents

All chemicals and reagents used in this study were purchased from Sigma (St. Louis, MO, USA) unless otherwise specified.

Plant material and preparation of the extract

The dry roots of *Angelica dahurica* Bentham et Hooker (ADBH) were obtained from Chungju Hospital of Korean Medicine, College of Korean Medicine, Semyung University, Jecheon, Republic of Korea. These roots were chopped to a length of 0.5 cm, dried in the shade, and powdered in a mechanical grinder. The pulverized roots were extracted with absolute ethanol, 70% ethanol, absolute methanol, 70% methanol, water, and boiling water at 60°C for 3 h, and the extraction was dried under a vacuum rotary evaporator (CCA-1110; EYELA, Tokyo, Japan).

Ten grams of dry roots were used for each group, and 2.172, 5.270, 3.109, 5.284, 2.831, and 5.695 g were extracted by absolute ethanol, 70% ethanol, absolute methanol, 70% methanol, water, and boiling water, respectively. The yield of the absolute ethanol, 70% ethanol, absolute methanol, 70% methanol, water, and boiling water groups were 21.7%, 52.7%, 31.1%, 52.8%, 28.3%, and 57.0% (w/w), respectively.

Anti-inflammatory assay

The RAW 264.7 cell line was purchased from the Korean Cell Line Bank (Seoul, Korea). These cells were maintained in RPMI 1640 medium supplemented with 10% FBS, 100 U/mL of penicillin, and 100 µg/mL of streptomycin. The cells were incubated at 37°C in a humidified atmosphere of 95% air and 5% CO₂.

The cytotoxicity of the samples on RAW 264.7 cells was tested in the following manner. The cells were seeded into 96-well plates at a density of 1×10^5 cells/

well, and after incubation for 18 h, the cells were exposed to RPMI medium along with samples at different concentrations for 24 h. The supernatant was removed from each well and 10 µL of MTT solution (5 mg/mL in phosphate-buffered saline) and 90 µL of the FBS-free medium was added to each well and incubated for 4 h at 37°C. Thereafter, the supernatant was sucked out and 200 µL of DMSO was added to each well. The plate was vibrated slightly for 10 min, and the MTT formazan was quantified by measuring absorbance at 490 nm using an enzyme-linked immunosorbent assay (ELISA) plate reader (ELx800, Bio-Tek, Winooski, VT, USA). The optical density of formazan formed in the control cells was considered to have 100% viability. Cell viability was expressed as a percentage of the control culture value. The cells were also incubated with lipopolysaccharides (LPS) (2 µg/mL) and cell viability in the presence of different extracts from *Angelicae Dahuricae Radix* was measured.

RAW 264.7 cells were plated in 96-well cell plates and incubated for 18 h. Thereafter, the cells were stimulated with LPS (2 µg/mL) in the presence or absence of samples at various concentrations for 24 h. Indomethacin served as a control. Aliquots of 100 µL of cell culture medium were mixed with 100 µL of Griess reagent (0.1% aqueous solution of *N*-(1-naphthyl)ethylenediamine dihydrochloride, 50 µL; 1% sulfanilamide [in 5% phosphoric acid], 50 µL), and the absorbance was determined at 550 nm using an ELISA plate reader (ELx800) (9).

RAW 264.7 cells (1×10^6) were grown in 6-well plates for 18 h, treated with various concentrations of samples for 30 min, followed by addition of LPS (2 µg/mL). After incubation for 24 h, total RNA of the cells was isolated with a TRIzol RNA isolation kit (Invitrogen, Carlsbad, CA, USA), reverse-transcribed to cDNA, and used as the template for PCR amplification. The forward and reverse primers were as follows: 5'-CACTCACGGCAAATTCAACGGCA-3' and 5'-GACTCCACGACATACTCAGCAC-3' for GAPDH, 5'-CCCTTCCGAAGTTTCTGGCAGCAG-3' and 5'-GGCTGTCAGAGCCTCGTGGCTTTGG-3' for inducible nitric oxide synthase (iNOS), 5'-TGGACGGACCCCAAAAGATG-3' and 5'-AGAAGGTGCTCATGTCTCTCA-3' for interleukin-1β (IL-1β), and 5'-CACTACATCCTGACCCACTT-3' and 5'-ATGCTCCTGCTTGAGTATGT-3' for cyclooxygenase-2 (COX-2). The amplified PCR products were separated on 1% agarose gel, which was then stained with ethidium bromide. It was then photographed with a Mini BIS Image Analysis System (DNR Bio-Imaging Systems Ltd., Jerusalem, Israel), and quantification of the

Fig. 1 (A) Cell viability of RAW 264.7 cells after incubation in the presence of different extracts from *Angelicae Dahuricae Radix* for 24 h. (B) Cell viability of RAW 264.7 cells after incubation with LPS in the presence of different extracts of *Angelicae Dahuricae Radix*. (C) Cell viability of RAW 264.7 cells in the presence of different extracts of *Angelicae Dahuricae Radix* after incubation with LPS for 30 min. Each value is expressed as mean $\pm$ SD ($n = 3$). #: Statistically significant differences were seen when compared with the control (non-treated group) ($P < 0.05$). *: There were statistically significant differences when compared with the 100 µg/mL group in each extraction method ($P < 0.05$).

iNOS, IL-1 β , and COX-2 expression levels were carried out using densitometric measurement.

Cytotoxic assay

The PC-3 human prostate cancer cell line, the A549 lung cancer cell line, KB keratin-forming tumor cell line, and HEK293 kidney cell line were purchased from the Korean Cell Line Bank (Seoul, Korea). Cells were maintained in DMEM medium supplemented with 10% FBS, 100 U/mL of penicillin, and 100 µg/mL of streptomycin. The cells were incubated at 37°C in a humidified atmosphere

of 95% air and 5% CO₂.

The cytotoxicity of samples on PC-3, A549, KB, and HEK293 cells was detected by MTT assay (9). Cells were seeded into 96-well plates and incubated with samples for 24 h for PC-3 and 24, 48, or 72 h for the A549, KB, and HEK293 cell lines. Thereafter, the supernatant was removed and 100 µL of MTT solution was added to each well and incubated for 4 h at 37°C. The supernatant was then sucked out and 200 µL of DMSO was added to each well. The amount of MTT formazan was quantified by measuring absorbance at 550 nm.

Fig. 2 (A) Nitric oxide inhibitory ability of different extracts of *Angelicae Dahuricae Radix* on LPS-stimulated RAW 264.7 cells. Indomethacin served as a control. (B) The effects of nitric oxide inhibitory ability of different extracts of *Angelicae Dahuricae Radix* after incubation with LPS for 30 min. #: Statistically significant differences were seen when compared with the control (non-treated group) ($P < 0.05$). *: There were statistically significant differences when compared with the 100 µg/mL group in each extraction method ($P < 0.05$).

Statistical Analysis

The data are reported as mean $\pm$ standard deviation (SD). A one-way analysis of variance (ANOVA) with post hoc test was performed to determine the differences between the groups using a commercially available program (SPSS 12 for Windows, SPSS Inc., Chicago, IL, USA). The level of significance was 0.05.

Results

Anti-inflammatory assay

The effects of *Angelicae Dahuricae Radix* on RAW 264.7 cells are presented in Fig. 1. The results showed that the water groups (100, 200, and 400 µg/mL) showed significant differences when compared to the non-loaded control ($P < 0.05$) (Fig. 1A). The cell viability of RAW 264.7 cells after incubation with LPS in the presence of different extracts from *Angelicae Dahuricae Radix* are shown in Fig. 1B. The effects of different extracts from *Angelicae Dahuricae Radix* after incubation with LPS for 30 min on cell viability of RAW 264.7 cells are presented in Fig. 1C.

Stimulation with LPS for 24 h led to a robust increase in nitric oxide production. However, *Angelicae*

Dahuricae Radix at 400 µg/mL concentration significantly suppressed nitric oxide by the LPS-stimulated RAW 264.7 cells in 70% ethanol, $25.6 \pm 0.4\%$; absolute ethanol, $89.7 \pm 2.0\%$; 70% methanol, $44.5 \pm 1.6\%$; absolute methanol, $83.1 \pm 1.6\%$; boiling water groups, $24.1 \pm 1.0\%$; and indomethacin group, 48.6 ± 3.8 ($P < 0.05$) (Fig. 2A). The highest decrease was noted in the absolute ethanol group, which along with the absolute methanol group, showed higher nitric oxide inhibitory effects than indomethacin ($P < 0.05$). Statistically significant decreases in nitric oxide production at 200 µg/mL concentration were noted only in the 70% ethanol, absolute ethanol, and 70% methanol, absolute methanol, boiling water groups, and indomethacin group, with the nitric oxide reductions being $12.5 \pm 0.9\%$, $47.3 \pm 1.7\%$, $20.8 \pm 1.5\%$, $45.1 \pm 0.4\%$, $20.9 \pm 0.5\%$, and $30.4 \pm 0.6\%$, respectively ($P < 0.05$). The effects of different extracts from *Angelicae Dahuricae Radix* on nitric oxide inhibitory ability cells after incubation with LPS for 30 min are shown in Fig. 2B.

The iNOS, IL-1 β , and COX-2 mRNA expression in the unstimulated RAW 264.7 cells was minimal, but their mRNAs were profoundly induced after treatment

Fig. 3 Effects of absolute ethanol extract of *Angelicae Dahuricae Radix* on nitric oxide synthase (iNOS), interleukin 1β (IL-1β), and cyclooxygenase-2 (COX-2) mRNA expression in LPS-stimulated RAW 264.7 cells. RAW 264.7 cells were treated with 1 μg/mL of LPS and different concentrations of absolute ethanol extract from *Angelicae Dahuricae Radix* for 24 h. The levels of iNOS, IL-1β, COX-2, and GAPDH mRNA were determined by a semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR).

Fig. 4 (A) Quantification of the iNOS expression level with densitometric measurement. (B) Quantification of the IL-1β expression level. (C) Quantification of the COX-2 expression level.

with LPS (Fig. 3). Pretreatment with absolute ethanol extract of *Angelicae Dahuricae Radix* suppressed the LPS-stimulated iNOS, IL-1β, and COX-2 expression. The suppression of inflammatory related genes increased with increasing concentration of *Angelicae Dahuricae Radix* extract (Fig. 4A-C).

Cytotoxic assay

The cell viability of PC-3 cells after incubation in the presence of different extracts from *Angelicae Dahuricae Radix* for 24 h are shown in Table 1.

The cell viability of A549 cells after incubation in the presence of different extracts from *Angelicae Dahuricae Radix* for 24 h, 48 h, and 72 h are also shown in Table 1. The results at 24 h showed that *Angelicae Dahuricae Radix* extract at 800 μg/mL concentration significantly reduced cellular viability in the water and boiling water groups when compared to the untreated control (cellular viability of 64.6 ± 1.3 and 92.2 ± 2.9 , respectively ($P < 0.05$)). However, statistically significant decreases in cellular viability at 400 μg/mL concentration was noted only in the water group (cellular viability of 72.1 ± 1.5 ($P < 0.05$)). The results at 48 h and 72 h showed trends that were similar to that of 24 h, i.e., a decrease of viability and longer incubation time. After 72 h, the water group exhibited the strongest effects ($87.8 \pm 1.0\%$, $79.2 \pm 2.5\%$, and $77.2 \pm 1.0\%$ at concentrations of 200, 400, and 800 μg/mL, respectively; control = $100.0 \pm 2.4\%$ ($P < 0.05$)).

The cell viability of KB cells and HEK293 cells after incubation in the presence of different extracts from *Angelicae Dahuricae Radix* for 24 h, 48 h, and 72 h are shown in Table 1.

Table 1 Cell viability of PC-3 cells, A549 cells, KB cells, and HEK293 cells after incubation with different extracts of *Angelicae Dahuricae Radix* for 24 h

Cells	Time	Concentration (µg/mL)	70% EtOH (%)	EtOH (%)	70% MeOH (%)	MeOH (%)	Water (%)	Boiling water (%)
PC-3 cells	24 h	200	103.5 ± 5.5	100.8 ± 2.5	98.7 ± 2.0	117.0 ± 2.3 [#]	96.2 ± 4.3	98.7 ± 1.8
		400	110.0 ± 2.8 ^{#,*}	110.2 ± 2.3 ^{#,*}	105.9 ± 3.7	107.9 ± 1.9 ^{#,*}	103.8 ± 3.9	100.6 ± 2.1
		800	116.4 ± 3.1 ^{#,*}	123.0 ± 2.0 ^{#,*}	113.9 ± 4.5 ^{#,*}	97.7 ± 2.7 [*]	113.4 ± 4.2 ^{#,*}	112.2 ± 2.3 ^{#,*}
A549 cells	24 h	200	104.6 ± 2.7	109.8 ± 1.6 [#]	101.1 ± 1.3	102.2 ± 2.2	73.0 ± 3.0 [#]	105.6 ± 2.6
		400	103.0 ± 3.3	111.5 ± 0.5 [#]	102.0 ± 0.7	102.9 ± 0.1	72.1 ± 1.5 [#]	96.3 ± 0.6 [*]
		800	100.6 ± 1.1	119.0 ± 0.2 ^{#,*}	98.2 ± 0.8 [*]	93.9 ± 2.3	64.6 ± 1.3 ^{#,*}	92.2 ± 2.9 ^{#,*}
	48 h	200	106.3 ± 1.0	109.1 ± 1.5 [#]	102.6 ± 1.3	102.0 ± 2.1	92.7 ± 1.9 [#]	105.9 ± 2.2
		400	102.1 ± 0.8	102.2 ± 3.6 [#]	101.8 ± 0.4	102.8 ± 0.2	88.0 ± 1.9 [#]	97.7 ± 0.4 [*]
		800	101.2 ± 0.5	107.5 ± 0.6 ^{#,*}	98.5 ± 1.0 [*]	97.6 ± 0.7	78.5 ± 3.3 ^{#,*}	93.0 ± 2.3 ^{#,*}
	72 h	200	100.9 ± 0.9	102.9 ± 1.0	98.5 ± 1.3	98.9 ± 2.1	87.8 ± 1.0 [#]	101.1 ± 0.9
		400	100.4 ± 1.6	103.1 ± 1.2	96.3 ± 5.6	98.5 ± 3.2	79.2 ± 2.5 ^{#,*}	100.6 ± 1.8
		800	91.9 ± 1.6 ^{#,*}	102.1 ± 2.4	93.2 ± 2.6	94.4 ± 0.9	77.2 ± 1.0 ^{#,*}	99.4 ± 1.6
KB cells	24 h	200	100.4 ± 3.6	103.6 ± 1.1	98.6 ± 2.5 [#]	103.1 ± 1.5	77.1 ± 0.5 [#]	86.5 ± 0.9 [#]
		400	95.0 ± 1.4	93.9 ± 2.0 [*]	93.4 ± 3.5	96.7 ± 1.3 [*]	73.9 ± 1.8 ^{#,*}	81.0 ± 0.4 ^{#,*}
		800	81.8 ± 1.1 ^{#,*}	76.1 ± 4.0 ^{#,*}	84.7 ± 1.0 ^{#,*}	71.8 ± 1.1 [*]	67.2 ± 0.2 ^{#,*}	72.8 ± 1.9 ^{#,*}
	48 h	200	95.0 ± 0.9 [#]	100.9 ± 3.9	97.9 ± 4.4	96.8 ± 0.6	81.8 ± 2.3 [#]	90.9 ± 1.0 [#]
		400	85.3 ± 1.5 ^{#,*}	84.2 ± 1.6 ^{#,*}	90.5 ± 0.7 [#]	84.7 ± 2.0 ^{#,*}	74.9 ± 1.0 ^{#,*}	83.6 ± 2.6 ^{#,*}
		800	71.5 ± 3.0 ^{#,*}	60.2 ± 1.4 ^{#,*}	79.4 ± 3.7 ^{#,*}	68.0 ± 3.1 ^{#,*}	67.2 ± 1.4 ^{#,*}	74.2 ± 2.5 ^{#,*}
	72 h	200	96.4 ± 1.0 [#]	95.8 ± 1.0 [#]	95.1 ± 2.4	84.2 ± 1.0 [#]	71.4 ± 1.0 [#]	76.4 ± 2.3 [#]
		400	85.1 ± 2.7 ^{#,*}	79.9 ± 2.4 ^{#,*}	90.7 ± 2.2 [#]	73.4 ± 1.2 ^{#,*}	65.5 ± 0.6 ^{#,*}	72.6 ± 1.4 [#]
		800	73.2 ± 0.9 ^{#,*}	47.8 ± 1.3 ^{#,*}	77.8 ± 4.1 ^{#,*}	55.0 ± 3.6 ^{#,*}	56.9 ± 2.5 ^{#,*}	66.1 ± 1.8 ^{#,*}
HEK293 cells	24 h	200	96.2 ± 1.7	95.1 ± 1.7	98.1 ± 4.0	93.9 ± 3.0	87.0 ± 1.8	85.7 ± 2.7
		400	92.2 ± 0.9	90.5 ± 0.7	92.0 ± 1.7	86.1 ± 1.3	76.6 ± 0.8	76.2 ± 0.2
		800	86.8 ± 1.1	84.6 ± 2.1	85.9 ± 1.3	81.2 ± 1.6	69.0 ± 2.2	68.6 ± 1.0
	48 h	200	97.1 ± 0.2	89.7 ± 4.5	99.3 ± 2.0	86.3 ± 2.1	89.6 ± 1.9	93.3 ± 3.9
		400	90.9 ± 2.1	77.3 ± 2.3	90.7 ± 2.2	79.3 ± 0.1	83.5 ± 2.2	88.6 ± 0.9
		800	83.4 ± 2.9	69.5 ± 3.0	80.7 ± 2.5	67.1 ± 3.0	75.4 ± 1.3	82.5 ± 0.2
	72 h	200	91.8 ± 0.9	79.6 ± 2.3	93.7 ± 2.7	72.3 ± 2.3	84.7 ± 1.5	80.2 ± 2.0
		400	81.8 ± 1.8	71.5 ± 1.3	83.4 ± 1.4	61.5 ± 1.6	81.1 ± 0.9	76.8 ± 1.4
		800	70.9 ± 1.6	55.7 ± 0.4	70.4 ± 1.6	46.9 ± 2.1	73.5 ± 1.7	74.3 ± 1.3

Each value is expressed as mean ± SD ($n = 3$).

[#]: Statistically significant differences were seen when compared to the control (non-treated group) at each time point ($P < 0.05$).

^{*}: There were statistically significant differences when compared with the 200 µg/mL group in each extraction method ($P < 0.05$).

Discussion

This study shows the effects of predetermined concentrations of different extracts of *Angelicae Dahuricae Radix* on mouse leukemic monocyte-macrophages, human prostate cancer cells, and human adenocarcinoma cells. In this experimental setting, the absolute ethanol extracts exhibited the strongest anti-inflammatory effects and the water extracts showed the highest cytotoxic effects.

This study clearly showed that the anti-inflammatory effects varied with the extraction solvent (methanol, ethanol, and water) and concentration. A previous *in vivo* study showed that methanol extracts of *Angelicae Dahuricae Radix* exhibited anti-inflammatory effects (8). In this study, we used the widely-used mouse leukemic monocyte macrophage model to evaluate the anti-inflammatory effects as inflammatory mediators such as nitric oxide are generated when the macrophage is stimulated with lipopolysaccharides (10). The previous study

showed that nitric oxide production was significantly suppressed by iNOS in a dose-dependent manner (8). This study clearly proved that the *Angelicae Dahuricae Radix*-mediated inhibition of nitric oxide production was a consequence of inhibition of iNOS, IL-1 β , and COX-2 and that the suppressive activity of *Angelicae Dahuricae Radix* was mediated via transcriptional levels. The nitric oxide inhibitory ability of different extracts from *Angelicae Dahuricae Radix* after incubation with LPS for 30 min suggested that the anti-inflammatory effects of ethanol were not simply due to the inhibition of LPS binding to the cells. Further studies are warranted to evaluate the underlying mechanisms for these anti-inflammatory effects.

Although many anti-cancer drugs have been developed, almost all of them are reported to have severe side effects on normal tissues and organs (11). Our previous study showed that no toxicity was seen for the mesen-

chymal stem cells in 0.001-100 µg/mL concentration of water extract of *Angelicae Dahuricae Radix* up to day 7 (12).

The PC-3 human prostate cancer cell line is widely used in prostate cancer research and is useful in investigating the biochemical changes in advanced prostatic cancer cells and assessing their response to chemotherapeutic agents (13). *Angelicae Dahuricae Radix* did not show statistically significant cytotoxic effects on PC-3 cells, and its extracts should not be recommended for advanced prostatic cancer. The A549 human adenocarcinoma cell line is squamous and is responsible for the diffusion of some substances. It is widely used as an *in vitro* model, especially for cytotoxicity (14). This study clearly showed that water extracts of *Angelicae Dahuricae Radix* produced statistically significant decreases in the cellular viability of A549 cells. A previous report suggested that the cytotoxic responses may be explained by imperatorin and coumarin, which form one of the major compounds of *Angelicae Dahuricae Radix* (2). Gas chromatography-mass spectrometry was used to determine several coumarins from *Angelicae Dahuricae Radix* (7). Further research may help understand the mechanisms underlying these cytotoxic effects.

Within the limitations of this study, *Angelicae Dahuricae Radix* extracts showed anti-inflammatory effects on the RAW 264.7 cell line and cytotoxic effects on A549 cells and KB cells, and these effects were influenced by the extraction methods. Absolute ethanol extracts showed the strongest anti-inflammatory effects while water extracts exhibited the highest cytotoxic effects in this experimental setting.

Conflict of interest

This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, Information and Communication Technology & Future Planning (NRF-2014R1A1A1003106). The authors report no conflicts of interest related to this study. The author does not have any financial interest in the companies whose materials are included in the article.

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