Natural Product Sciences
27(4) : 228-233 (2021)
https://doi.org/10.20307/nps.2021.27.4.228

Chemical Constituents from *Agrimonia pilosa* with Inhibitory Activity against Interleukin 1β Production via NLRP3 and NLRC4 Inflammasomes

Piseth Nhoek, Hee-Sung Chae, Chae-Yeong An, Pisey Pel, Young-Mi Kim, and Young-Won Chin*

College of Pharmacy and Research Institute of Pharmaceutical Sciences, Seoul National University, 1 Gwanak-lo, Gwanak-gu, Seoul 08826, Republic of Korea

Abstract – Bioactivity-guided fractionation by preliminary screening using interleukin-1β production in lipopolysaccharides (LPS)-induced J774A.1 cell line led to the isolation of fourteen structures including chromone, isocoumarins, flavanoids, and triterpenes from the aerial part of *Agrimonia pilosa* Ledeb. All structures were determined by measuring their spectroscopic data and comparing their spectroscopic data with the literatures. All the isolates were tested for their inhibitory activities against interleukin-1β production in LPS-induced J774A.1 cell. Of the tested compounds, (*S*)-(+)-5,7-dihydroxy-2-(1-methylpropyl)chromone (**1**), agrimonolide-6-*O*-*β*-D-glucopyranoside (**5**), agrimonolide-6-*O*-*α*-L-arabinofuranosyl-(1→6)-*β*-D-glucopyranoside (**6**), and catechin (**10**) were found to be active. Furthermore, compound **1** suppressed the protein expressions of NLRP3 and NLRC4 in murine macrophage.

Keywords – *Agrimonia pilosa*, interleukin-1β, chromone, isocoumarin, inflammation, NLRP3, NLRC4, inflammasome

Introduction

Inflammasomes are complex proteins capable of recognizing exo- and endogenous dangers.[1] Up to date, several inflammasomes such as nucleotide-binding domain and leucine-rich repeat receptor (NLR) family pyrin domain-containing 3 (NLRP3), NLR family pyrin domain-containing 1 (NLRP1), absent in melanoma 2 (AIM 2), and NLR family CARD domain-containing protein 4 (NLRC4) have been reported,[2] and NLRP3 is the most well studied inflammasome among them.[3] NLRP3 inflammasome is composed of a sensor (NLRP3), an adaptor, apoptosis associated speck-like protein containing a caspase recruitment domain (ASC) and an effector protein (caspase 1).[4] Two different steps, priming and activation, are required for the activation of NLRP3 inflammasome. The priming step (signal 1) is that toll-like receptor 4 (TLR4) triggers NF-κB-mediated NLRP3, and pro-IL-1β expression. The activation step (signal 2) is regulated by pathogen-associated molecular patterns (PAMPs) and danger-associated molecular patterns (DAMPs), resulting in NLRP3 complex assembly and caspase 1 activation.[5,6] The activation of caspase 1 leads to cleavage of pro-IL-1β into active biological state.[7]

NLRC4 inflammasome plays an important role as the platform for caspase 1 activation and IL-1β secretion.[8] NLRC4 inflammasome is a flagellin cytosolic sensor and needs apoptosis inhibitory protein (NAIP).[9] NAIP proteins recognize the ligands, and then recruit NLRC4 proteins into inflammasome.[10] NLRC4 is linked to caspase 1 by adapter protein ASC, which leads to the maturation of IL-1β.[11]

Agrimonia pilosa Ledeb. (Rosaceae) is a perennial herbaceous plant,[12] has been traditionally used for the treatment of abdomen pain, sore, headaches,[13] diarrheas,[14] gastrointestinal, gynecological, and genitourinary diseases[15] in China, Japan, and Korea. The previous phytochemical studies on the aerial part of *A. pilosa* have reported the presence of isocoumarins, flavonoids, phenolic glycosides,[16,17] tannins, and triterpenes,[18,19] as the chemical constituents. Individual constituents or extracts of *A. pilosa* have revealed a variety of pharmacological properties including anti-allergic activities,[20] anti-inflammatory,[19,21-24] anti-tumor,[25] and anti-viral activities.[26,27]

Even though the anti-inflammatory effects of the individual constituents from *A. pilosa* have been carried

Dedicated to Prof. Jinwoong Kim of the Seoul National University for his pioneering works on Pharmacognosy.

*Author for correspondence
Prof. Young-Won Chin, College of Pharmacy and Research Institute of Pharmaceutical Sciences, Seoul National University, 1 Gwanak-lo, Gwanak-gu, Seoul 08826, Republic of Korea
Tel: +82-2-880-7859; E-mail: ywchin@snu.ac.kr

out,[21-24] the inflammasome-mediated IL-1β production inhibitory effects of the isolated chemical constituents from this plant have not been explored. Thus, in the present study, we investigated the CH_2Cl_2-soluble extract which was selected by the preliminary bioassay using LPS-induced J774A.1 cell and the compounds isolated from the CH_2Cl_2 and EtOAc-soluble fractions were evaluated for their inhibitory effects of IL-1β release in LPS-induced J774A.1.

Experimental

General experimental procedure – NMR spectra were measured on a Varian 400 spectrometer (Varian, CA, USA) NMR spectrometers. High-resolution mass spectrometric data were obtained using a Waters Xevo G2 Q-TOF mass spectrometer (Waters, MA, USA). Semi-preparative highpressure liquid chromatography (HPLC) was conducted with a Gilson 321 pump and Gilson 172 diode array detector (Gilson, Madison, WI, USA) equipped with HPLC columns [250 × 10 mm, Luna column, 5 μm] (Phenomenex, Seoul, Korea) and an Acclaim Polar Advantage 2 column [250 × 21.2 mm, Thermo Scientific, 5 μm] (Life Technologies Korea LCC, Seoul, South Korea). Medium Pressure Liquid Chromatography (MPLC) was performed by using Isolera One (Biotage, Hengoed, United Kingdom). Water was purified with a Milli-Q system (Waters). Column chromatography was conducted on RP-C_{18} silica gel (Cosmosil, Kyoto, Japan) Sephadex LH-20 (GE Healthcare, Uppsala, Sweden). Thin-layer chromatography (TLC) analysis was performed on silica gel 60 F254 plates (Merck, Darmstadt, Germany). The spots were visualized by spraying with 10% aqueous H_2SO_4.

Plant material – The dried aerial parts of *A. pilosa* Ledeb were purchased from Daelim Korean Medical Market (Chungbuk, Republic of Korea) and identified by Dr. Hee-Sung Chae. A voucher specimen (CYWSNU-AP0021) was deposited at the College of Pharmacy, Seoul National University, Republic of Korea.

Extraction and isolation – The dried aerial parts of *A. pilosa* Ledeb (4.3 kg) were extracted three times with CH_3OH at room temperature and evaporated *in vacuo*. The crude extract (530.6 g, AP) was suspended in H_2O and sequentially partitioned with organic solvents to give 29.5 g of *n*-hexane-soluble extract, 22.7 g of CH_2Cl_2-soluble extract, 69.4 g of EtOAc-soluble extract, 74.0 g of *n*-butanol-soluble extract and water-soluble extract. The CH_2Cl_2-soluble extract (20.7 g, APC) was chromatographed over a silica column chromatography (CC) (5 × 90 cm, 500 g) using gradient mixtures of *n*-hexane–EtOAc (100:0 → 1:1), following by mixtures of $CHCl_3$–CH_3OH (20:1 → 1:1), and finished by 100% CH_3OH, providing 13 sub-fractions (APC1–APC13). APC10 (120.0 mg) was subjected to HPLC [250 × 10 mm, Luna column, 5 μm] eluted with CH_3CN–H_2O (75:25) with a flow rate of 1.5 mL/min in isocratic mode for 30 min and then the column was flushed by 100% CH_3CN for 5 min, to afford compound **4** (t_R 15.3 min, 17.0 mg). Sub-fraction APC11 (1.4 g) was purified by medium pressure liquid chromatography (MPLC) using a reversed-phase (RP) column with gradient mixtures of CH_3OH–H_2O (10:90 → 100:0) to afford 15 sub-fractions (APC11A–APC11T). The combined sub-fractions APC11H (7.0 mg) and APC11I (11.0 mg) was subjected to HPLC [250 × 21.2 mm, Thermo Scientific, 5 μm] eluted with CH_3OH–H_2O (35:75) with a flow rate of 3 mL/min for 20 min in isocratic elution mode, to furnish compound **2** (t_R 16:2 min, 1.4 mg). A HPLC [250 × 21.2 mm, Thermo Scientific, 5 μm] method using CH_3OH–H_2O (55:45) in isocratic mode (3 mL/min) for 20 min was used to separate compound **3** (t_R 15.8 min, 3.3 mg) from APC11K (20.0 mg). APC11O (98.0 mg) was separated on HPLC [250 × 10 mm, Luna column, 5 μm] by an isocratic elution (1.5 mL/min) with CH_3CN–H_2O (60:40) for 30 min, providing compounds **13** (t_R 26.1 min, 4.4 mg) and **14** (t_R 30.6 min, 11.9 mg). APC12 (3.02 g) was fractionated by MPLC RP-C_{18} column chromatography eluted with gradient mixtures of CH_3OH–H_2O (10:90 → 100:0), to afford 15 sub-fractions (APC12-1–APC12-15). The separation of APC12-9 (60.0 mg) was performed on HPLC [250 × 10 mm, Luna column, 5 μm] using a mixture of CH_3OH–H_2O (80:20) in isocratic elution (1.5 mL/min) for 15 min and sequentially flushed the column by 100% CH_3OH for 5 min, furnishing compound **1** (t_R 7.2 min, 1.1 mg). Compound **5** (t_R 10.2 min, 22.0 mg) was purified from APC12-14 (54.0 mg) by HPLC [250 × 21.2 mm, Thermo Scientific, 5 μm] eluted with CH_3OH–H_2O (60:40) in isocratic elution (3 mL/min) for 15 min. The EtOAc-soluble fraction (AgE, 69.3 g) was fractionated by a column chromatography by using Diaion HP20 resin with CH_3OH–H_2O (0:100 → 100:0) and following by 100% acetone as solvent to afford 10 sub-fractions (AgE1–AgE10). AgE3 (4.0 g) was further subjected to a silica column chromatography with gradient mixtures of $CHCl_3$–CH_3OH (100:0 → 0:100) to obtain 15 sub-fractions (AgE3-1–AgE3-15). The separation of AgE3-11 (314.2 mg) was conducted on HPLC [250 × 10 mm, Luna column, 5 μm] with a flow rate of 1.5 mL/min with CH_3CN–H_2O (25:75) for 12 min, (30:70) for 12 min, (50:50) for 16 min, and followed by 100% CH_3CN for 5

min, providing compounds **10** (t_R 10.2 min, 2.3 mg), **9** (t_R 30.2 min, 1.3 mg), and **8** (t_R 32.2 min, 11.2 mg). AgE3-12 (149.8 mg) was separated on HPLC [250 × 10 mm, Luna column, 5 μm] with a flow rate of 1.5 mL/min using $CH_3CN–H_2O$ (18:82) for 20 min, (20:80) for 40 min, (40:60) for 20 min, and then 100% CH_3CN for 5 min to give impure compound **7** (t_R 47.5 min, 4.4 mg). The impure compound **7** was further purified by HPLC [250 × 10 mm, Luna column, 5 μm] eluted with $CH_3CN–H_2O$ (55:45) in isocratic elution (1.5 mL/min) for 15 min, furnishing compound **7** (t_R 13.3 min, 0.6 mg). Four sub-fractions (AgE4-1–AgE4-4) were fractionated from AgE4 (3.09 g) by MPLC RP-C_{18} eluted with $CH_3OH–H_2O$ (10:90 → 40:60). The purification of compound **6** (t_R 10.2 min, 3.1mg) from AgE4-3 (74.0 mg) was conducted on HPLC [250 × 10 mm, Luna column, 5 μm] eluted with $CH_3OH–H_2O$ (60:40) in isocratic elution mode with a flow rate of 1.5 mL/min for 15 min and finishing by 100% CH_3CN for 5 min. AgE6 (4.70 g) was separated in the same manner with AgE4, to yield 4 sub-fractions (AgE6-1–AgE6-4). AgE6-1 (624.0 mg) was purified over Sephadex LH 20 column eluted with $CH_3OH–H_2O$ (1:1) to afford 18 sub-fractions including compounds **11** (4.4 mg) and **12** (15.2 mg).

Cell culture and chemical reagents – Murine macrophages, RAW 264.7 and J774A1, were obtained from the Korean Research Institute of Bioscience and Biotechnology (Republic of Korea), and grown in RPMI medium containing 10% fetal bovine serum and 100 U/mL penicillin/streptomycin sulfate. Cells were incubated in a humidified 5% CO_2 atmosphere at 37 °C. DMEM, penicillin, and streptomycin were purchased from Hyclone (Logan, UT, USA). Bovine serum albumin (BSA), lipopolysaccharide (LPS) were purchased from Sigma (St. Louis, MO, USA). Anti-mouse IL-1β antibody and biotinylated anti-mouse IL-1 antibody were purchased from Invitrogen (Invitrogen; Thermo Fisher Scientific, Inc., Carlsbad, CA, USA). NLRP3, NLRP4, caspase-1, AIM2, β-actin, antibodies were purchased from Cell Signaling Technology, Inc. (Danvers, MA).

Immunoblot analysis – Protein expression was assessed by Western blotting according to standard procedures. Briefly, J77A.1 cells were cultured in 60-mm culture dishes (2 × 10^6 cells/mL), following by pretreatment various concentrations of compound (4 and 20 μM). Cells were washed twice in ice cold PBS (pH 7.4), the cell pellets were resuspended in lysis buffer on ice for 15 min, and the cell debris was then removed by centrifugation. Protein concentration was determined using protein assay reagent (Bio-Rad) according to the manufacturer's instructions. Protein (20–30 μg) was mixed 1:1 with 5 × sample buffer (20% glycerol, 4% SDS, 10% 2-ME, 0.05% bromophenol blue, and 1.25 M Tris [pH 6.8]), loaded onto 8% SDS-polyacrylamide gel electrophoresis gels, and run at 150 V for 90 min. Cellular proteins were transferred onto ImmunoBlot polyvinylidene difluoride membranes (Bio-Rad) using a Bio-Rad semi-dry transfer system according to the manufacturer's instructions. The membranes were then incubated overnight with the respective primary antibody (diluted 1:500–1:1000) in Tris-buffered saline containing 5% skim milk and 0.1% Tween 20. The following day, the blots were washed three times with Tris-buffered saline (0.1% Tween 20) and incubated for 1 h with an horseradish peroxidase conjugated secondary anti-IgG antibody (diluted 1:2000–1:20,000). The blots were washed again three times with Tris-buffered saline (0.1% Tween 20), and immunoreactive bands were developed using the chemiluminescent substrate ECL Plus (Milipore, Billerica, MA, USA). Images were acquired by using a ChemiDoc Imaging system (ChemiDoc™ XRSsystem with Image Lab™ software 3.0; Bio-Rad, Hercules, CA, USA).

Measurement of interleukin 1β – Cells were seeded at 1 × 10^6 cells/mL per well in 24 well tissue culture plates and pretreated with the indicated concentration of compounds for 30 min before stimulation. After 6 h, the supernatant was decanted into a new micro centrifuge tube, and the amount of IL-1β was determined using the ELISA kit according to the procedure described by the manufacturer (ThermoFisher Scientific, Waltham, MA, USA). All subsequent steps took place at room temperature, and all standards and samples were assayed in duplicate.

Result and Discussion

The methanol extract of *A. pilosa* was found to inhibit the release of IL-1β in LPS-induced J774A.1. Therefore, the organic solvent soluble fractions (hexane, dichloromethane, ethyl acetate, butyl alcohol and water-soluble extracts*)* were measured by the same screening assay. The dichloromethane-soluble fraction exhibited the inhibitory activity against the release of interleukin 1β. Consequencely, dichloromethane-soluble extract was chosen to search for compounds (Fig. 1). Additionally, ethyl acetate-soluble fraction was conducted to investigate for new compounds as well as the chemical constituents from this plant.

Fourteen chemical compounds (**1**–**14**) were isolated and characterized from the aerial parts of *A. pilosa* (Fig.

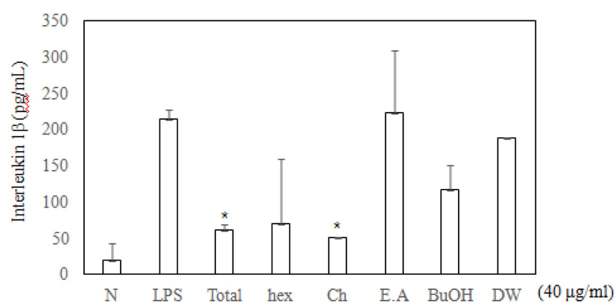

Fig. 1. The effect of methanolic extract (Total), hexane (hex), dichloromethane (Ch), ethyl acetate (E.A), butanol (BuOH) and water (DW) fractions of A. pilosa on IL-1β release.

2). The structures were confirmed by measuring NMR spectroscopic data and by comparing with the reported values as (*S*)-(+)-5,7-dihydroxy-2-(1-methylpropyl)chromone (**1**),[28] loliolide (**2**),[29] desmethylagrimonolide (**3**),[30] agrimonolide (**4**),[31] agrimonolide-6-*O*-*β*-D-glucopyranoside (**5**),[32] agrimonolide-6-*O*-*α*-L-arabinofuranosyl-(1→6)-*β*-D-glucopyranoside (**6**),[33] 1-(2-methylbutyryl)phloroglucinol-glucopyranoside (**7**),[34,35] undulatoside A (**8**),[36] urachromone B (**9**),[37] catechin (**10**),[38] taxifolin-3-*O*-*β*-D-glucopyranoside (**11**),[39] quercetin-3-*O*-*β*-D-glucopyranoside (**12**),[40] 1*β*,2*β*,3*β*,19*α*-tetrahydroxyurs-12-en-28-oic acid (**13**),[41] and 1*α*,2*β*,19*β*-trihydroxy-3-oxo-12-ursen-28-oic acid (**14**).[42]

Anti-inflammatory effects of *A. pilosa* have been documented in several publications,[19,21-24] including the inflammasome-mediated anti-inflammatory effect of *A. pilosa* extract.[43] However, the effects of the individual constituents from *A. pilosa* has not been explored. Hence, this study focuses on inflammasome-mediated anti-inflammatory effects of the isolated compounds with IL-1β production using LPS-induced J774A. 1 cell.

All isolated compounds **1−14** were investigated for the inhibitory activity of IL-1β release (Fig. 3). Of tested compounds, compound **1**, agrimonolide-6-*O*-*β*-D-glucopyranoside (**5**), agrimonolide-6-*O*-*α*-L-arabinofuranosyl, 6-*O*-*β*-D-glucopyranoside (**6**) and catechin (**10**) (Fig. 3) were found to inhibit the release of IL-1β at a concentration of 40 μM compared with the LPS-treated group. Catechin (**10**) was known to inhibit IL-1β secretion via modulation of NLRP inflammasome activation.[44] Also, agrimonolide (**4**), an aglycone of **5** and **6**, was reported to reduce IL-1β secretion[28] but in the LPS-induced J774A cells, agrimonolide (**4**) did not reduce IL-1β secretion. Instead, compounds **5** and **6**, glycosides of agrimonolide, suppressed IL-1β secretion.

Among these active compounds, compound **1** was selected for further investigation because this chromone-type compound has never been explored in the inflammasome related IL-1β production.

Since IL-1β production results mainly from the activation of NLRP3 and NLRC4 inflammasome, then

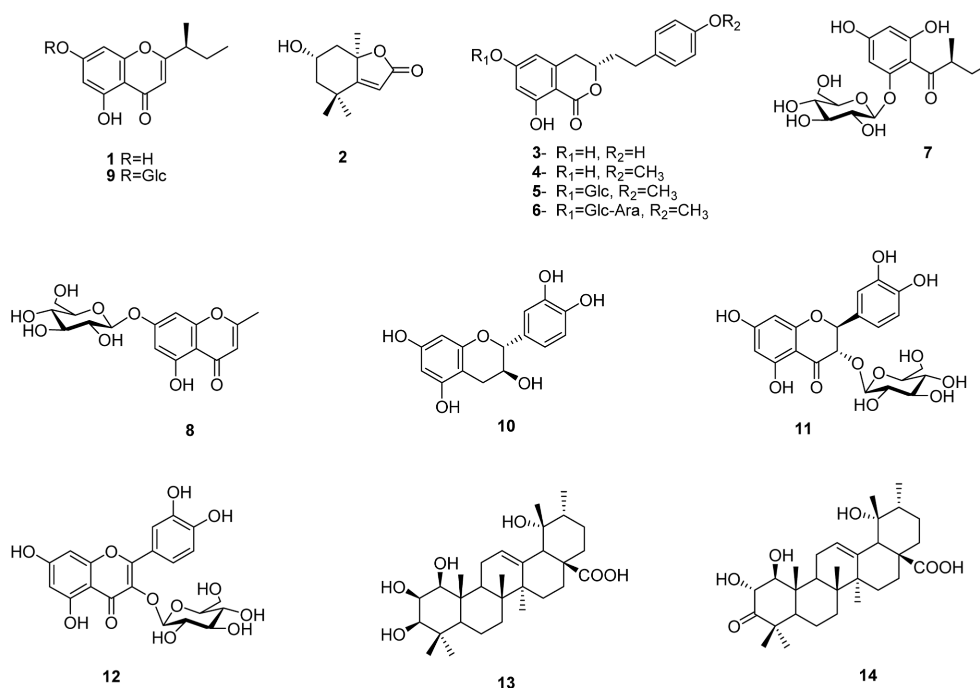

Fig. 2. Chemical structures isolated from *A. pilosa.*

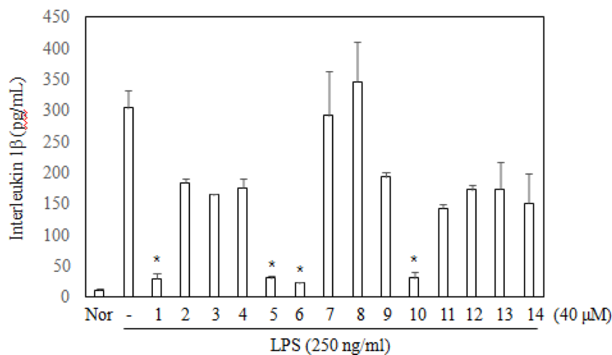

Fig. 3. Effect of *A. pilosa* on the release of interleukin 1β in LPS-induced J774A.1 by ELISA. (A) Effect of compounds from *A. pilosa* on the release of interleukin 1β. Statistical significance: *P<0.05 as compared to the LPS treated cell. Values shown are the mean ± S.E. of duplicate determinations from three separate experiments. (B) The effects of compound **1** on the inflammasome pathway in LPS-stimulated J774A.1 cells. J774A.1 cells were treated with the indicated concentrations of compound 1 (10 and 20 μM) for 30 minutes prior to being incubated with LPS for 6 hours. Whole cell lysates were then analyzed by Western blotting.

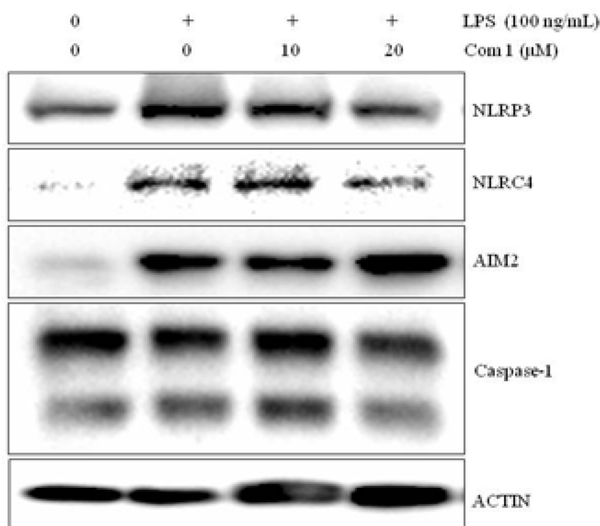

Fig. 4. The effects of compound **1** on the inflamasome pathway in LPS-stimulated J774A.1 cells. J774A.1 cells were treated with the indicated concentrations of compound **1** (10 and 20 μM) for 30 minutes prior to being incubated with LPS for 6 hours. Whole cell lysates were then analyzed by Western blotting.

we evaluated the effect of compound **1** (Fig. 4). Compound **1** slightly decreased NLRP3 and NLRC4 protein expression with 20 μM in J774A.1. The NLRP3 inflammasome is activated in a two-step process. First, nuclear factor (NF)-κB signaling is induced through adenosine monophosphate (AMP)-mediated activation of toll-like receptor (TLR) 4, resulting in increased expression of NLRP3. Next, indirect activation of NLRP3 occurs by a multitude of signals (lysosomal-damaging environmental factors and endogenous, and mitochondrial damage), leading to complex assembly and activation of caspase-1. Interestingly, in J774.1, caspase-1 and AIM 2 which plays a primordial role in inflammasome formation, compound **1** did not change caspase-1 and AIM2 protein expression as compared to only LPS-treated group. Thus, this compound seemed to inhibit IL-1β production via NLRP3 and NLRC4 inflammasomes.

In conclusion, compounds **1**, **5**, **6**, and **10** were found to inhibit the release of IL-1β. Among these active compounds, compound **1** decreased NLRP3 and NLRC4 protein expression. This finding might provide the potential of *A. pilosa* extract in the treatment of inflammatory-related diseases.

Acknowledgment

This study was supported by a grant (NRF-2019 R1A2C2009053) from the National Research Foundation of Korea (NRF), funded by the Korean government (MSIT).

Conflicts of Interest

The authors declare no conflict of interest.

References

(1) Sharma, D.; Kanneganti, T. D. *J. Cell Biol*. **2016**, *213*, 617-629.
(2) Guo, Q.; Wu, Y.; Hou, Y.; Liu, Y.; Liu, T.; Zhang, H.; Fan, C.; Guan, H.; Li, Y.; Shan, Z.; Teng, W. *Front. Immunol*. **2018**, *9*, 1197.
(3) Moossavi, M.; Parsamanesh, N.; Bahrami, A.; Atkin, S. L.; Sahebkar, A. *Mol. Cancer* **2018**, *17*, 158.
(4) Swanson, K. V.; Deng, M.; Ting, J. P. Y. *Nat. Rev. Immunol*. **2019**, *19*, 477-489.
(5) Yang, Y.; Wang, H.; Kouadir, M.; Song, H.; Shi, F. *Cell Death Dis*. **2019**, *10*, 128.
(6) Malik, A.; Kanneganti, T. D. *J. Cell Sci*. **2017**, *130*, 3955-3963.
(7) Kelley, N.; Jeltema, D.; Duan, Y.; He, Y. *Int. J. Mol. Sci*. **2019**, *20*, 3328-3351.
(8) Mao, K.; Chen, S.; Wang, Y.; Zeng, Y.; Ma, Y.; Hu, Y.; Zhang, H.; Sun, S.; Wu, X.; Meng, G.; Pei, G.; Sun, B. *J. Immunol*. **2015**, *194*, 1867-1873.
(9) Lee, E.; Karki, R.; Kanneganti, T. D. *Cell Stress* **2018**, *2*, 144-146.
(10) Verma, V.; Gupta, S.; Kumar, P.; Yadav, S.; Dhanda, R. S.; Gaind, R.; Arora, R.; Frimodt-MØller, N.; Yadav, M. *Front. Microbiol*. **2019**, *10*, 1-15.
(11) Franchi, L.; Eigenbrod, T.; Munoz-Planillo, R.; Nunez, G. *Nat. Immunol*. **2009**, *10*, 241-247.
(12) Jin, X.; Song, S.; Wang, J.; Zhang, Q.; Qiu, F.; Zhao, F. *Exp. Ther. Med.* **2016**, *12*, 499-505.
(13) Park, S. H.; Sim, Y. B.; Kang, Y. J.; Lee, J. K.; Lim, S. S.; Suh, H. W. *Korean J. Physiol. Pharmacol*. **2012**, *16*, 119-123.
(14) Miyamoto, K.; Koshiura, R.; Ikeya, Y.; Taguchi, H. *Chem. Pharm. Bull.* **1985**, *33*, 3977-3981.

(15) Liu, X.; Zhu, L.; Tan, J.; Zhou, X.; Xiao, L.; Yang, X.; Wang, B. *BMC Complement. Altern. Med.* **2014**, *14*, 12.
(16) Taira, J.; Ohmine, W.; Ogi, T.; Nanbu, H.; Ueda, K. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 1766-1769.
(17) Park, E. J.; Oh, H.; Kang, T. H.; Sohn, D. H.; Kim, Y. C. *Arch. Pharm. Res.* **2004**, *27*, 944-946.
(18) Wang, Y.; Liu, M.; Zheng, L.; Yin, L.; Xu, L.; Qi, Y.; Ma, X.; Liu, K.; Peng, J. *J. Sep. Sci.* **2012**, *35*, 1977-1984.
(19) Ma, J. H.; Jiang, Q. H.; Chen, Y.; Nie, X. F.; Yao, T.; Ding, L. Q.; Zhao, F.; Chen, L. X.; Feng, Q. *Nat. Prod. Commun.* **2015**, *10*, 2041-2044.
(20) Kim, J. J.; Jiang, J.; Shim, D. W.; Kwon, S. C.; Kim, T. J.; Ye, S. K.; Kim, M. K.; Shin, Y. K.; Koppula, S.; Kang, T. B.; Choi, D. K.; Lee, K. H. *J. Ethnopharmacol*. **2012**, *140*, 213-221.
(21) Chen, L.; Teng, H.; Fang, T.; Xiao, J. *Phytomedicine* **2016**, *23*, 846-855.
(22) Jung, C. H.; Kim, J. H.; Park, S.; Kweon, D. H.; Kim, S. H.; Ko, S. G. *Immunol. invest*. **2010**, *39*, 159-170.
(23) Nho, K. J.; Chun, J. M.; Kim, H. K. *J. Ethnopharmacol.* **2011**, *138*, 358-363.
(24) Wang, X.; Wang, H.; Zhang, C.; Zhang, K. *Afr. J. Tradit. Complement. Altern. Med*. **2013**, *10*, 475-479.
(25) Koshiura, R.; Miyamoto, K.; Ikeya, Y.; Taguchi, H. *Jpn. J. Pharmacol*. **1985**, *38*, 9-16.
(26) Shin, W. J.; Lee, K. H.; Park, M. H.; Seong, B. L. *Microbiol. Immunol*. **2010**, *54*, 11-19.
(27) Kwon, J. E.; Lee, Y. G.; Kang, J. H.; Bai, Y. F.; Jeong, Y. J.; Baek, N. I.; Seo, Y. J.; Kang, S. C. *Bioorg. Chem*. **2019**, *93*, 103320.
(28) Qiu, D.; Zhou, M.; Lin, T.; Chen, J.; Wang, G.; Huang, Y.; Jiang, X.; Tian, W.; Chen, H. *J. Nat. Prod*. **2019**, *82*, 1072-1080.
(29) Yang, X.; Kang, M. C.; Lee, K. W.; Kang, S. M.; Lee, W. W.; Jeon, Y. J. *ALGAE* **2011**, *26*, 201-208.
(30) Kato, H.; Li, W.; Koike, M.; Wang, Y.; Koike, K. *Phytochemistry* **2010**, *71*, 1925-1929.
(31) Huang, Q.; Chen, L.; Teng, H.; Song, H.; Wu, X.; Xu, M. *J. Funct. Foods* **2015**, *19*, 487-494.
(32) Taira, J.; Nanbu, H.; Ueda, K. *Food Chem*. **2009**, *115*, 1221-1227.
(33) Kim, H. W.; Park, J.; Kang, K. B.; Kim, T. B.; Oh, W. K.; Kim, J.; Sung, S. H. *J. Nat. Prod.* **2016**, *79*, 2376-2383.
(34) Zhang, Y. J.; Abe, T.; Tanaka, T.; Yang, C. R.; Kouno, I. *Chem. Pharm. Bull*. **2002**, *50*, 841-843.
(35) Bohr, G.; Gerhäuser, C.; Knauft, J.; Zapp, J.; Becker, H. *J. Nat. Prod*. **2005**, *68*, 1545-1548.
(36) Peng, B.; Bai, R. F.; Li, P.; Han, X. Y.; Wang, H.; Zhu, C. C.; Zeng, Z. P.; Chai, X. Y. *J. Asian Nat. Prod. Res.* **2016**, *18*, 59-64.
(37) Chen, X. Q.; Li, Y.; Cheng, X.; Wang, K.; He, J.; Pan, Z. H.; Li, M. M.; Peng, L. Y.; Xu, G.; Zhao, Q. S. *Chem. Biodivers.* **2010**, *7,* 196-204.
(38) Jeon, S. H.; Chun, W.; Choi, Y. J.; Kwon, Y. S. *Arch. Pharm. Res*. **2008**, *31*, 978-982.
(39) Dübeler, A.; Voltmer, G.; Gora, V.; Lunderstädt, J.; Zeeck, A. *Phytochemistry* **1997**, *45*, 51-57.
(40) Han, J. T.; Bang, M. H.; Chun, O. K.; Kim, D. O.; Lee, C. Y.; Baek, N. I. *Arch. Pharm. Res*. **2004**, *27*, 390-395.
(41) Kouno, I.; Baba, N.; Ohni, Y.; Kawano, N. *Phytochemistry* **1988**, *27*, 297-299.
(42) Lee, T. H.; Lee, S. S.; Kuo, Y. C.; Chou, C. H. *J. Nat. Prod*. **2001**, *64*, 865-869.
(43) Li, C.; W.; Sui, J.; Zhou, Y.; Chen, W. *Acta Biochim. Biophys. Sin*. **2021**, *53*, 1342-1353.
(44) Jhang, J. J.; Lu, C. C.; Ho, C. Y.; Cheng, Y. T.; Yen, G. C. *J. Agric. Food Chem*. **2015**, *63*, 7343-7352.

Received November 9, 2021
Revised December 6, 2021
Accepted December 7, 2021