Prev. Nutr. Food Sci. 2025;30(1):68-80
https://doi.org/10.3746/pnf.2025.30.1.68
ISSN 2287-8602

Effects of *Astragalus membranaceus* Leaf Extract on Allergic Inflammation in Immune Cell Lines

Perleidulam Bunddulam[1], Mizuki Nakamura[1], Anuu Zorig[1], Yu Hinata[2], Mikako Takasugi[3], Chao-Hui Feng[1], Toshitsugu Sato[1], and Hirofumi Arai[1]

[1]School of Regional Innovation and Social Design Engineering, Kitami Institute of Technology, Hokkaido 090-8507, Japan
[2]Laboratory of Seeds Discovery and Development, Hokkaido 089-4300, Japan
[3]Department of Life Science, Kyushu Sangyo University, Fukuoka 813-8503, Japan

ABSTRACT: *Astragalus membranaceus* is a perennial plant belonging to the Fabaceae family that is widely distributed across northern Asia. *A. membranaceus* roots have been used as a traditional medicinal herb because of their numerous health benefits. However, the physiological properties of *A. membranaceus* leaves remain unknown. During allergic reactions, chemical mediators are released from mast cells, which trigger immediate hypersensitivity and subsequent macrophage-mediated inflammation. In the present study, we investigated the inhibitory effects of *A. membranaceus* leaf extract (AMLE) on allergic and inflammatory responses in immune cell lines. The results showed that AMLE suppressed the release of histamine and leukotriene B_4 from stimulated mast cells during allergic response by inhibiting the influx of calcium ions and downregulating the expression of 5-lipoxygenase. Moreover, AMLE suppressed the release of nitric oxide, prostaglandin E_2, and cytokines from stimulated macrophages during inflammatory response. These effects were attributed to the suppression of mRNA and protein expressions of inducible nitric oxide synthase and microsomal prostaglandin E synthase-1 and the inhibition of the nuclear translocation of nuclear factor kappa-light-chain-enhancer of activated B cells. Compared with the *A. membranaceus* root extract, AMLE had more potent inhibitory effects. Quercetin and kaempferol, the predominant polyphenolic compounds present in AMLE, suppressed the release of chemical mediators during allergic and inflammatory responses in immune cells. These results suggest that *A. membranaceus* leaves have a therapeutic potential for alleviating allergic inflammation and that polyphenols are the primary bioactive constituents of *A. membranaceus* leaves.

Keywords: allergy, *Astragalus membranaceus*, inflammation, macrophages, mast cells

INTRODUCTION

Astragalus membranaceus is a perennial herb belonging to the Fabaceae family that is primarily distributed across northern Asia (Tan et al., 2019; D'Avino et al., 2023). *A. membranaceus* roots have long been used as a key pharmaceutical component in traditional Chinese medicine. Their pharmacological effects, which are mainly attributed to astragalosides, have been studied to determine their potential to modulate immune function and antioxidant properties (Adesso et al., 2018; Chen et al., 2021). By contrast, the physiological activities of the aerial parts of *A. membranaceus*, which are only consumed as tea in China, remain unknown.

Recently, the prevalence of allergic diseases, particularly type I allergies (e.g., pollen hypersensitivity), has increased (Song et al., 2015; Almatroudi et al., 2021; Yagi et al., 2024). Allergies are adverse immune responses to harmless substances, including pollen. During allergic response, basophils and mast cells play pivotal roles. These cells are activated when immunoglobulin (Ig) E conjugated to Fcε receptor I on the cellular membrane is cross-linked with a specific allergen, which triggers downstream intracellular signal transduction (Kawasaki et al., 1994; Siraganian, 2003). This stimulation induces the influx of intracellular calcium ions (Ca^{2+}), leading to the release of histamine by degranulation and causing allergic symptoms such as a runny nose. During signal transduction, 5-lipoxygenase (LOX) oxidizes arachidonate released from the cellular membrane to produce leukotrienes (LTs). LTB_4 induces the migration of immune cells, including macrophages (Wang et al., 2021), to the site of an allergic reaction (Oh et al., 2010) and causes chronic allergic symptoms (Lotfi et al., 2020). The chemical me-

Received 2 December 2024; Revised 9 December 2024; Accepted 12 December 2024; Published online 28 February 2025
Correspondence to Hirofumi Arai, E-mail: araihrfm@mail.kitami-it.ac.jp

diators that enhance vascular permeability during allergic responses promote inflammation, wherein macrophages play a central role (Björk et al., 1982; Nicoletti et al., 2012; Ashina et al., 2015). Macrophages are activated when external substances, including lipopolysaccharides (LPS), from bacteria interact with toll-like receptors (TLRs) on cellular membranes. This stimulation initiates intracellular signaling pathways, which produce proinflammatory cytokines, including interleukin (IL)-1β and IL-6 (Lee et al., 2016; Li et al., 2023a). The pathway of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) is essential in this process as its activation regulates the transcription of inflammation-related genes (Liu et al., 2017; Verghese et al., 2018). Moreover, macrophages produce nitric oxide (NO) and prostaglandin (PG) E_2, which amplify the inflammatory response (Lee et al., 2018).

Recently, natural compounds from plants, including polyphenols, have attracted attention as they exhibit physiological activities that alleviate various diseases (Kumar and Pandey, 2013; Castell et al., 2014; Ko et al., 2018). Many natural bioactive compounds are used clinically and have the potential to replace conventional Western medicines (Chin et al., 2006; Zhang et al., 2013). Previous studies have demonstrated the potential of functional foods to mitigate allergic symptoms and inflammation with minimal side effects (Kawai et al., 2007). In the present study, the antiallergic and anti-inflammatory activities of the *A. membranaceus* leaf extract (AMLE) were investigated using immune cell lines. Furthermore, the mechanisms underlying the inhibitory effects of AMLE on allergic and inflammatory reactions were evaluated, and the causative agents were identified.

MATERIALS AND METHODS

Chemicals

Astragaloside IV and calycosin were purchased from Chemscene and Cayman Chemical Co., respectively. Kaempferol and quercetin were obtained from Tokyo Chemical Industry, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) was purchased from FUJIFILM Wako Pure Chemicals. All reagents used in this study were of analytical grade.

Preparation of the *A. membranaceus* extract

A. membranaceus was cultivated in Rikubetsu, Hokkaido, Japan. The leaves and roots of *A. membranaceus* plants were air-dried and finely chopped. A portion of the sample (18 g) was stirred in 1 L of water at 80°C for 20 min. Subsequently, the extract was filtered. To maximize the yield, the extraction process was repeated using the remaining residue. The extract solution was lyophilized to obtain AMLE and *A. membranaceus* root extract (AMRE) powders. The AMLE and AMRE yields were 39.1% and 13.9%, respectively.

Determination of total polyphenols

The Folin-Ciocalteu method was used to quantify the total polyphenols in AMLE and AMRE (Chapin, 1921). Briefly, a 200-μL aliquot of each sample dissolved in water was mixed with 200 μL of 1 N Folin-Ciocalteu reagent (Nacalai Tesque) and incubated at 25°C for 3 min. Subsequently, the mixture was added with 200 μL of 10% sodium carbonate. Then, it was allowed to react in the dark at 30°C for 30 min. The absorbance of the resulting solution was measured at 760 nm. Moreover, the total polyphenol content was calculated using a gallic acid calibration curve prepared under the same experimental conditions and expressed as gallic acid equivalents (mg GAE).

Antioxidant activity assay

DPPH assay was used to evaluate the antioxidant activities of AMLE and AMRE based on their radical scavenging abilities (Zorig et al., 2021). An appropriate amount of the sample was mixed with 100 μM DPPH in 75% ethanol and then reacted in the dark at 30°C for 30 min. The reaction mixture was centrifuged at 20,000 *g* for 5 min, and the absorbance of the resulting supernatant was measured at 517 nm.

Flavonol aglycone analysis

Reversed-phase high-performance liquid chromatography (RP-HPLC) was used to analyze the flavonol aglycones in AMLE in accordance with the method of a previous study (Qiu et al., 2023). The sample was hydrolyzed with 0.65 M HCl at 90°C for 1 h. The flavonol aglycones in the hydrolysate were injected into an InertSustain C18 column (5 μm, 4.6×250 mm, GL Science) maintained at 40°C. Separation was achieved using a mobile phase of 0.5% H_3PO_4 (A) and methanol (B) at a flow rate of 0.7 mL/min. The gradient elution program was as follows: 40% – 60% B from 0 min to 10 min, isocratic at 60% B from 10 min to 21 min, 60% – 40% B from 21 min to 23 min, and isocratic at 40% B from 23 min to 38 min. The absorbance of the eluent was measured at 370 nm.

Cell culture

The rat basophilic leukemia cell line (RBL-2H3) and mouse mast cell line (PB-3c) were supplied by the JCRB Cell Bank. The mouse macrophage cell line (RAW 264) was provided by the RIKEN BioResource Research Center. RBL-2H3 cells were cultured in Eagle's Minimal Essential Medium (MEM; FUJIFILM) supplemented with 10% fetal bovine serum (FBS; HyClone Laboratories), 100 units/mL of penicillin, and 100 μg/mL of strepto-

mycin (FUJIFILM). PB-3c cells were cultured in Roswell Park Memorial Institute (RPMI)-1640 medium (FUJIFILM) containing 1 mM sodium pyruvate, 2 mM L-glutamine, and 25 mM 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid supplemented with 10% FBS, 1% non-essential amino acids (Gibco), 4 μL/L of 2-mercaptoethanol, 2 μg/L of IL-3 (FUJIFILM), 100 units/mL of penicillin, and 100 μg/mL of streptomycin. RAW 264 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM; FUJIFILM) supplemented with 10% FBS, 100 units/mL of penicillin, and 100 μg/mL of streptomycin. All cell cultures were maintained under standard conditions at 37°C in a humidified atmosphere containing 5% CO_2.

The trypan blue dye exclusion method, a widely accepted technique for determining cell viability, was used to assess the sample cytotoxicity to RBL-2H3 and PB-3c cells. Meanwhile, the Cell Counting Kit-8 (Dojindo) was used to evaluate the sample cytotoxicity to RAW 264 cells (Zhao et al., 2017).

Histamine release assay

The inhibitory effects of AMLE and AMRE on histamine release from RBL-2H3 cells were evaluated in accordance with previously reported methods (Matsuo et al., 1997; Qiu et al., 2023). RBL-2H3 cells were sensitized with mouse anti-2,4-dinitrophenyl (DNP) IgE monoclonal antibody (Yamasa) in the medium at a density of 4×10^5 cells/well in 24-well microplates for 20 h. Then, the cells were rinsed twice with phosphate-buffered saline (PBS) (pH 7.4) and incubated with various concentrations of samples in 450 μL of Tyrode buffer (pH 7.2) comprising 137 mM NaCl, 2.7 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, 12 mM $NaHCO_3$, 0.4 mM NaH_2PO_4, and 5.6 mM glucose supplemented with 0.05% bovine serum albumin (BSA) (Sigma-Aldrich) at 37°C for 10 min. To induce cell stimulation, 50 μL of 2 μg/mL DNP-BSA (Millipore) was added in Tyrode buffer at 37°C for 20 min. To determine the total histamine content in cytoplasmic granules and baseline histamine release without stimulation, 50 μL of 5% Triton X-100 and Tyrode buffer without DNP-BSA were added, respectively. A 100-μL aliquot of the culture supernatant was mixed with 50 μL of 200 mM *N*-acetyl-L-cysteine and 50 μL of 8 μM 1-methylhistamine as an internal standard. The histamine concentration in the mixtures was analyzed by RP-HPLC using a Shodex ODP-50-4E column (5 μm, 4.6×250 mm; Showa Denko) maintained at 50°C (von Vietinghoff et al., 2006). The analytes were isocratically eluted with a methanol/water mixture (35:65, v/v) containing 30 mM $Na_2B_4O_7$ and 0.2 mM *o*-phthalaldehyde at a flow rate of 0.7 mL/min. The fluorescence of the derivatives in the eluate was detected using an excitation and emission wavelength of 340 and 450 nm, respectively.

Leukotriene B_4 production assay

The inhibitory effects of AMLE and AMRE on LTB_4 production in PB-3c cells were evaluated in accordance with a previously reported method (Takasugi et al., 2018). The PB-3c cells were cultured with 50 μM of arachidonate in the medium for 48 h. After the cells were rinsed twice with PBS, 4×10^6 cells were incubated with various concentrations of samples in 180 μL of Tyrode buffer at 37°C for 10 min. To induce cell stimulation, 20 μL of 10 μM calcium ionophore (A23187, Merck) was added at 37°C for 20 min. To terminate the stimulation, 200 μL of an acetonitrile/methanol mixture (30:25, v/v) containing 1 mM ascorbic acid, 2 mM ethylenediaminetetraacetic acid (EDTA), and 1 μM PGB_2 (Cayman Chemical) as an internal standard was added. The reaction mixture was centrifuged at 20,000 *g* at 4°C for 15 min, and the LTB_4 concentration in the supernatant was determined by RP-HPLC using an octadecylsilane column (5 μm, 6.0×150 mm; YMC-Pack ODS-A, YMC) maintained at 40°C. The analytes were isocratically eluted with an acetonitrile/methanol/water mixture (25:45:30, v/v/v) containing 5 mM CH_3COONH_4 at a flow rate of 1.0 mL/min. The absorbance of the eluate was measured at 280 nm.

Measurement of cytoplasmic calcium ions

The intracellular Ca^{2+} concentration in RBL-2H3 cells during stimulation was measured using the Calcium Kit II-Fluo 4 (Dojindo Laboratories) (Zorig et al., 2021). RBL-2H3 cells were sensitized with anti-DNP IgE at a density of 3×10^4 cells/well in 96-well microplates at 37°C for 20 h. Various concentrations of samples and 5 μg/mL of Fluo 4-AM (a Ca^{2+}-sensitive fluorescent dye) were added to the cells and incubated at 37°C for 1 h. Cell stimulation was induced by DNP-BSA, and the fluorescence intensity (excitation wavelength, 485 nm; emission wavelength, 520 nm) was continuously monitored at 37°C using a fluorescence microplate reader (Wallac 1420 ARVO MX/Light, PerkinElmer).

Nitric oxide and prostaglandin E_2 release assay

RAW 264 cells were seeded into 24-well microplates at a density of 4×10^5 cells/well and allowed to adhere for 2 h. The cells were incubated with various concentrations of samples in 495 μL of phenol red-free medium at 37°C for 30 min. Cell stimulation was induced by adding 5 μL of 100 μg/mL LPS (Sigma-Aldrich), and the cells were maintained at 37°C. The NO in the culture supernatant was measured after 24 h using the NO_2/NO_3 Assay Kit-FX with 2,3-diaminonaphthalene (Dojindo Laboratories). After 6 h, the PGE_2 in the culture supernatant was determined using an enzyme-linked immunosorbent assay (ELISA) kit with a monoclonal antibody (Cayman Chemicals).

Measurement of inflammatory cytokines

LPS was used to induce the stimulation of RAW 264 cells, as described previously. The IL-1β concentration in the culture supernatant was determined using an ELISA kit (R&D Systems) after 24 h of stimulation. Meanwhile, the IL-6 concentration in the culture supernatant was determined using an ELISA kit (Mabtech) after 6 h of stimulation.

Analysis of mRNA

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) was performed using the SYBR Green Fast Advanced Cells-to-Ct Kit (Thermo Fisher Scientific) to measure the expression levels of 5-LOX, mRNA in RBL-2H3 cells, as well as inducible nitric oxide synthase (iNOS) mRNA, and microsomal prostaglandin E synthase-1 (mPGES-1) mRNA in RAW 264 cells. The stimulated cells were lysed in the presence of DNase I, and complementary DNA was synthesized via reverse transcription in accordance with the manufacturer's instructions. RT-qPCR was performed using the LightCycler 96 System (Roche) with SYBR Green as the detection dye. The thermal cycling conditions comprised an initial enzyme activation step at 95°C for 10 min, followed by 45 amplification cycles comprising denaturation at 95°C for 3 s and annealing/extension at 60°C for 30 s. After the reaction, melting curve analysis was performed to ensure amplification specificity and the presence of a single product. The Primer-BLAST online tool was used to design the primer sequences used for RT-qPCR (Table 1). The relative gene expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method with normalization to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as the internal control gene.

Table 1. Primer sequences for reverse transcription-quantitative polymerase chain reaction

Gene		Sequence (5′-3′)
5-LOX	Forward	CCCCACGGGGACTACATAGA
	Reverse	TCGGGCCAATTTTGCACATC
iNOS	Forward	CGGCAAACATGACTTCAGGC
	Reverse	GCACATCAAAGCGGCCATAG
mPGES-1	Forward	GCCAACGACATGGAGACAATC
	Reverse	ATGTATCCAGGCGATCAGAGG
GAPDH	Forward	TGTGTCCGTCGTGGATCTGA
	Reverse	TTGCTGTTGAAGTCGCAGGAG

5-LOX, 5-lipoxygenase; iNOS, inducible nitric oxide synthase; mPGES-1, microsomal prostaglandin E synthase-1; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

Analysis of proteins

The protein expression levels of iNOS and mPGES-1 in RAW 264 cells were assessed using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) followed by Western blot analysis in accordance with previous studies (Laemmli, 1970; Zorig et al., 2021). The stimulated cells in a 24-well microplate were rinsed twice with cold tris-buffered saline (TBS) (pH 7.4) containing 1 mM EDTA and lysed using radioimmunoprecipitation assay lysis buffer (ATTO Corporation) supplemented with 1% protease inhibitor. Then, the lysates were incubated on ice for 15 min, collected with a cell scraper, and centrifuged at 20,000 *g* at 4°C for 20 min. The resulting supernatant was mixed with Laemmli Sample Buffer (Bio-Rad Laboratories) and heated at 95°C for 5 min. The proteins were separated using SDS-PAGE with Any kD TGX gels (Bio-Rad) and transferred onto polyvinylidene difluoride membranes. The membranes were blocked using Intercept Blocking Buffer (LI-COR) for 1 h, rinsed with TBS containing 0.05% Tween-20, and shaken for 2 h with the primary antibodies. The antibodies used targeted iNOS (rabbit polyclonal, GeneTex), mPGES-1 (mouse polyclonal, Abcam), or β-actin (mouse monoclonal, clone 8H10D10; Cell Signaling Technology), each prepared in Can Get Signal Immunoreaction Enhancer Solution (TOYOBO). After incubation with the primary antibodies, the membranes were rinsed with 0.05% Tween-TBS and shaken for 1 h with the secondary polyclonal antibodies conjugated with IRDye 680RD or 800CW (LI-COR) specific to mouse or rabbit IgG. The immunoreactive proteins were detected by near-infrared fluorescence at 680 and 800 nm using the Odyssey CLx Imaging System (LI-COR).

Analysis of NF-κB translocation

Nuclear fractions were extracted from stimulated RAW 264 cells using the NE-PER Nuclear and Cytoplasmic Extraction Reagents Kit (Thermo Fisher Scientific) in accordance with the manufacturer's instructions. As described previously, the isolated nuclear proteins were analyzed by SDS-PAGE and Western blot with a primary polyclonal rabbit antibody specific for NF-κB p65 (Thermo Fisher Scientific).

Statistical analysis

All experiments were repeated multiple times to ensure reproducibility in order to confirm the reliability of the results. All experimental data (n=3) were expressed as the mean±standard deviation. The Tukey-Kramer multiple comparison test or Student's *t*-test was used to perform group comparisons using the Mac Statistical Analysis software (version 3.0, Esumi). Statistical significance was considered at $P<0.05$.

RESULTS

Polyphenol content and radical scavenging ability

AMLE had a significantly higher polyphenol content (51.5

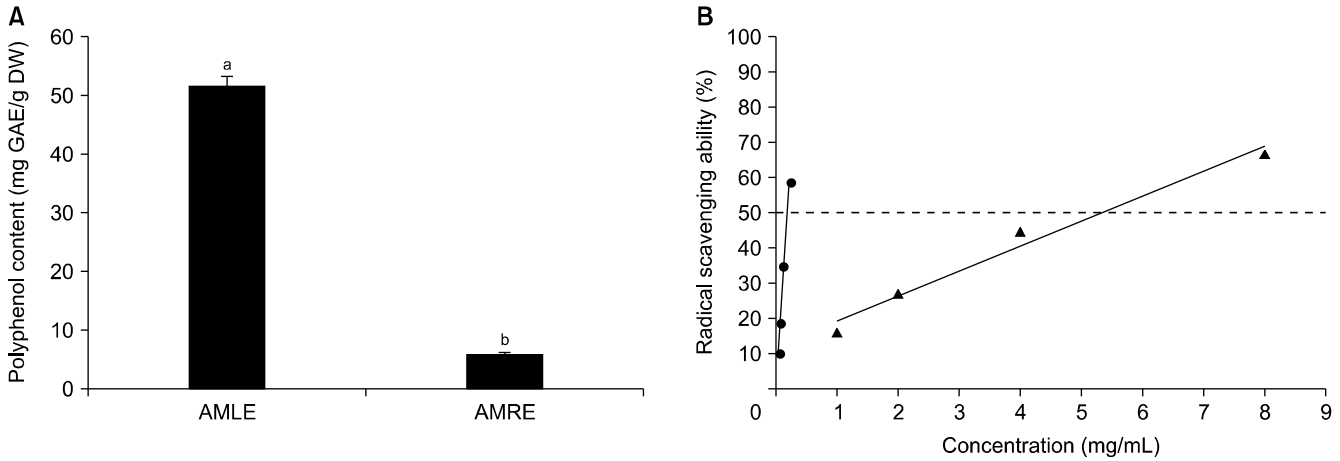

Fig. 1. Polyphenol contents and radical scavenging abilities of *Astragalus membranaceus* leaf extract (AMLE) and *A. membranaceus* root extract (AMRE). (A) Polyphenol content. The results are expressed as milligram gallic acid equivalents (GAE). (B) Radical scavenging ability. AMLE (●) and AMRE (▲). Values are presented as the mean±SD (n=3). Different letters (a, b) indicate a significant difference (P<0.05) between groups.

mg GAE/g dry weight) than AMRE (5.8 mg GAE/g dry weight) (Fig. 1A). Moreover, AMLE had a stronger DPPH radical scavenging ability [50% inhibitory concentration (IC_{50})=0.16 mg/mL] than AMRE (IC_{50}=5.34 mg/mL) (Fig. 1B).

Release of chemical mediators in allergic cell response

The effects of AMLE and AMRE on the release of chemical mediators during allergic response are shown in Fig. 2. AMLE significantly suppressed the release of histamine from stimulated RBL-2H3 cells at 1.0 mg/mL (Fig. 2A), whereas AMRE tended to suppress the release of histamine. AMLE and AMRE significantly suppressed the production of LTB_4 in stimulated PB-3c cells at 1.0 mg/mL (Fig. 2B), with the former showing a markedly stronger inhibitory activity than the latter. AMLE inhibited the release of histamine at 0.5, 1.0, and 2.0 mg/mL in a concentration-dependent manner (Fig. 2C). In addition, AMLE inhibited the production of LTB_4 at 0.2, 0.5, and 1.0 mg/mL in a concentration-dependent manner (Fig. 2D). The results of the trypan blue dye exclusion assay confirmed that neither AMLE nor AMRE was toxic

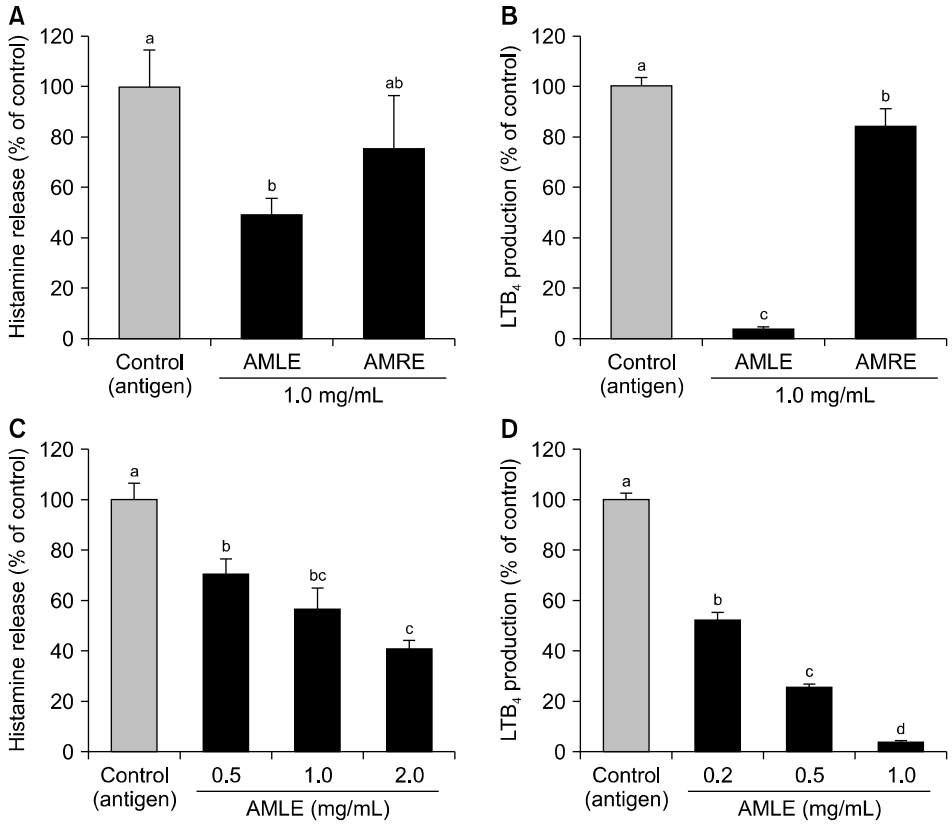

Fig. 2. Effects of *Astragalus membranaceus* leaf extract (AMLE) and *A. membranaceus* root extract (AMRE) on the release of chemical mediators in the allergic cell response. (A) Release of histamine from stimulated RBL-2H3 cells. (B) Leukotriene (LT) B_4 production by stimulated PB-3c cells. (C) Dose-dependent inhibitory effects of AMLE on the release of histamine. (D) Dose-dependent inhibitory effects of AMLE on the production of LTB_4. Values are presented as the mean±SD (n=3). Different letters (a-d) indicate significant differences (P<0.05) between groups. RBL-2H3, rat basophilic leukemia cell line; PB-3c, mouse mast cell line.

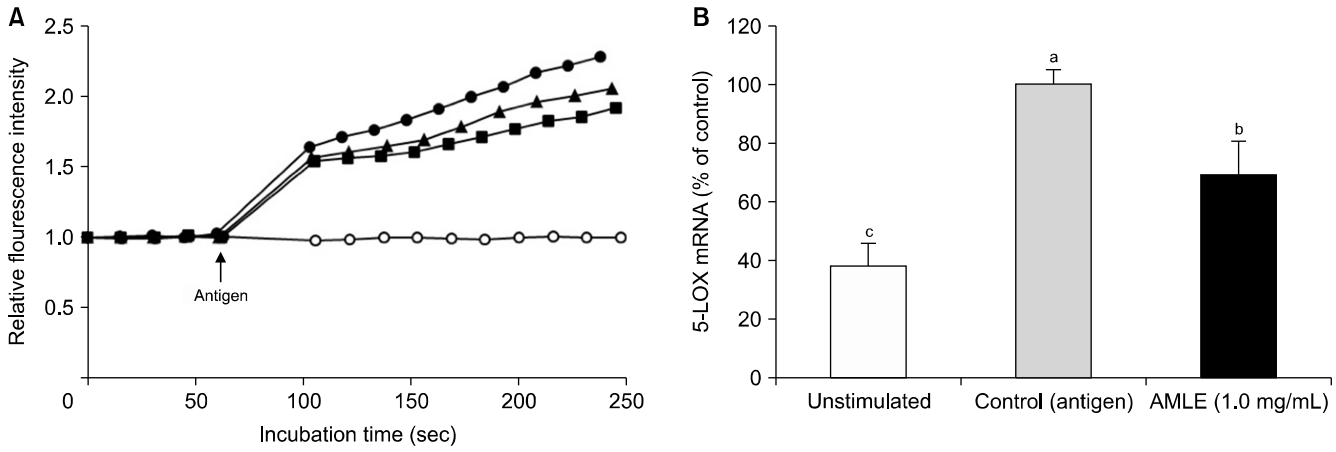

Fig. 3. Effects of *Astragalus membranaceus* leaf extract (AMLE) on intracellular calcium ion influx and 5-lipoxygenase (5-LOX) expression in allergic response. (A) Time course analysis of cytoplasmic calcium ion concentrations in RBL-2H3 cells. Unstimulated (○), control (●), 0.5 mg/mL of AMLE (▲), and 2.0 mg/mL of AMLE (■). (B) 5-LOX mRNA expression in RBL-2H3 cells. The values were normalized to the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase. Values are presented as the mean±SD (n=3). Different letters (a-c) indicate significant differences (P<0.05) between groups. RBL-2H3, rat basophilic leukemia cell line.

to RBL-2H3 and PB-3c cells up to 2.0 and 1.0 mg/mL, respectively (Supplementary Fig. 1).

Intracellular Ca^{2+} influx and 5-lipoxygenase expression in allergic response

The effects of AMLE on the changes in cytoplasmic Ca^{2+} concentration in the stimulated RBL-2H3 cells are shown in Fig. 3A. In the control group, the relative fluorescence intensity increased progressively after the stimulation (indicated by the arrow), whereas no significant changes were observed without stimulation. However, this stimulation-induced increase was suppressed with AMLE treatment at concentrations of 0.5 and 1.0 mg/mL in a concentration-dependent manner. The effects of AMLE on 5-LOX mRNA expression in stimulated RBL-2H3 cells are shown in Fig. 3B. Antigen stimulation increased 5-LOX mRNA levels; however, this expression was significantly inhibited by AMLE treatment (1.0 mg/mL).

Release of chemical mediators in inflammatory cell response

The effects of the *A. membranaceus* extracts on NO and PGE_2 release during inflammatory response are shown in Fig. 4A and 4B, respectively. AMLE significantly suppressed the release of NO and PGE_2 from stimulated RAW 264 cells at 0.2, 0.5, and 1.0 mg/mL in a concentration-dependent manner, whereas AMRE exhibited no effects at 1.0 mg/mL. The results of cell viability assay confirmed that AMLE and AMRE were not toxic to RAW 264 cells up to 1.0 mg/mL for 24 h (Supplementary Fig. 1).

Expression levels of chemical mediator synthesis enzymes in inflammatory cell response

The effects of AMLE on the mRNA expression levels of chemical mediator synthesis enzymes in LPS-stimulated RAW 264 cells are shown in Fig. 5. AMLE significantly inhibited the expression of iNOS (Fig. 5A) and mPGES-1

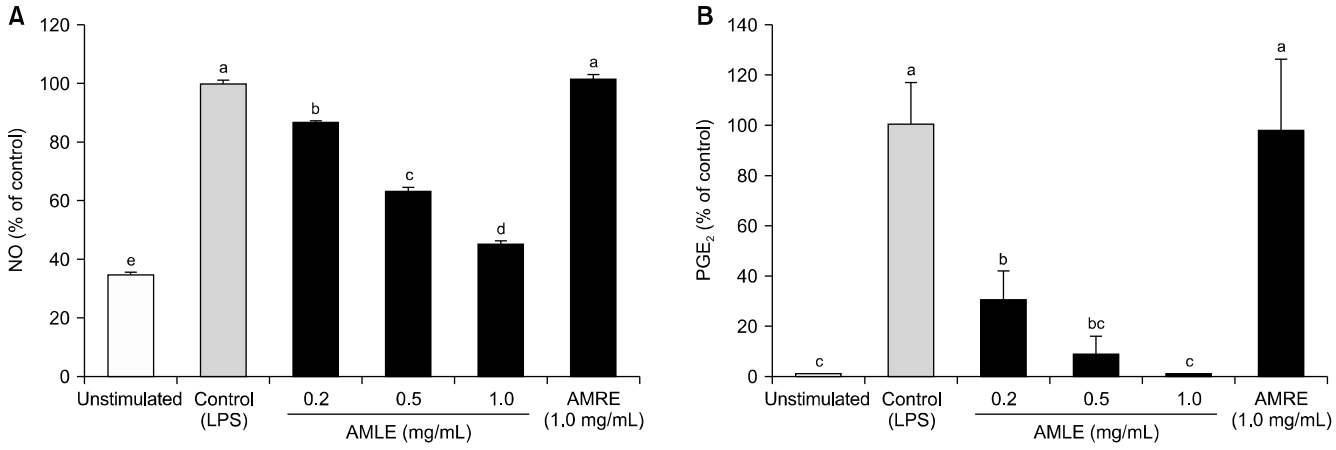

Fig. 4. Effects of *Astragalus membranaceus* leaf extract (AMLE) and *A. membranaceus* root extract (AMRE) on the release of chemical mediator in inflammatory cell response. (A) Nitric oxide (NO) release from RAW 264 cells. (B) Prostaglandin E_2 (PGE_2) from RAW 264 cells. Values are presented as the mean±SD (n=3). Different letters (a-e) indicate significant differences (P<0.05) between groups. RAW 264, mouse macrophage cell line.

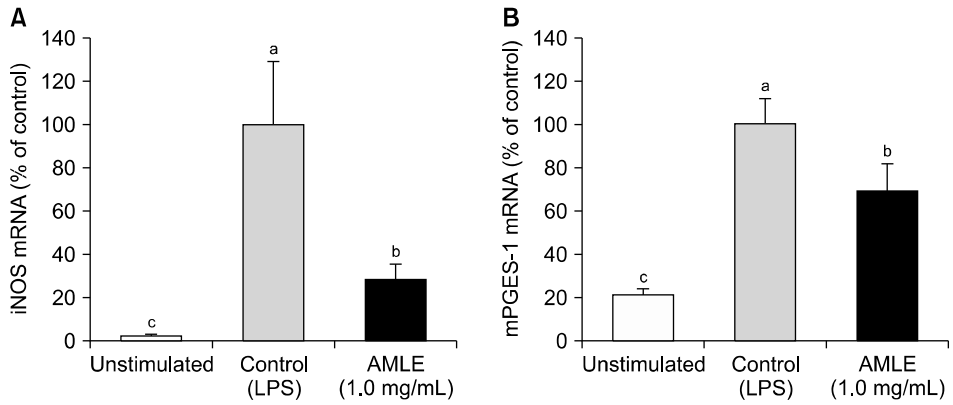

Fig. 5. Effects of *Astragalus membranaceus* leaf extract (AMLE) on the mRNA expression of chemical mediator synthesis enzymes in inflammatory response. (A) Inducible nitric oxide synthase (iNOS) in RAW 264 cells. (B) Microsomal prostaglandin E synthase-1 (mPGES-1) in RAW 264 cells. The values were normalized to glyceraldehyde-3-phosphate dehydrogenase. Values are presented as the mean±SD (n=3). Different letters (a-c) indicate significant differences (P<0.05) between groups. RAW 264, mouse macrophage cell line; LPS, lipopolysaccharides.

(Fig. 5B) at 1.0 mg/mL. The effects of AMLE on the protein expression levels of chemical mediator synthesis enzymes in LPS-stimulated RAW 264 cells are shown in Fig. 6. The results of Western blot analysis showed that AMLE (1.0 mg/mL) reduced the intensity of the iNOS band, corresponding to a molecular weight of 131 kDa (Fig. 6A, lane 2), compared with the control (lane 1). Based on triplicate experiments, the relative band intensities of iNOS normalized to β-actin at 42 kDa as a loading control for the total protein are shown in Fig. 6B. AMLE significantly inhibited iNOS protein expression. Similarly, AMLE treatment decreased the mPGES-1 band detected at 17 kDa (Fig. 6C) and significantly inhibited mPGES-1 protein expression (Fig. 6D).

Release of cytokines and nuclear translocation of NF-κB in inflammatory cell response

The effects of AMLE on the release of the inflammatory cytokines IL-1β and IL-6 from LPS-stimulated RAW 264 cells are shown in Fig. 7A and 7B, respectively. AMLE treatment at 0.5 and 1.0 mg/mL significantly inhibited the LPS-induced expression of IL-1β and IL-6 in RAW 264 cells in a concentration-dependent manner. The effects of AMLE on the nuclear translocation of NF-κB in LPS-stimulated RAW 264 cells are shown in Fig. 7C. AMLE treatment at 1.0 mg/mL significantly inhibited NF-κB nuclear translocation.

Effects of major *A. membranaceus* leaf compounds on the release of chemical mediators in allergic and inflammatory cell responses

The effects of major *A. membranaceus* leaf compounds (e.g., astragaloside IV, calycosin, kaempferol, and quercetin) on the release of histamine, LTB_4, NO, and PGE_2 from the stimulated immune cell lines are shown in Fig. 8A–8D, respectively. Astragaloside IV exhibited no inhibitory effects on the release of any chemical mediators at 40 μM. Kaempferol and quercetin significantly suppressed the release of histamine from stimulated RBL-2H3 cells at 40 μM, with the latter showing a stronger effect than the former. Calycosin had no inhibitory effects on the release of histamine at 40 μM. Kaempferol

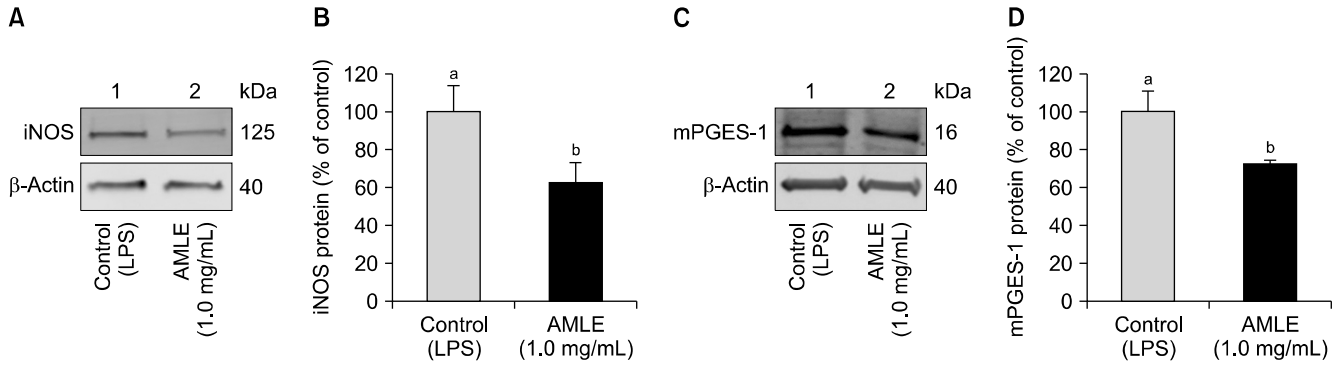

Fig. 6. Effects of *Astragalus membranaceus* leaf extract (AMLE) on the protein expression of chemical mediator synthesis enzymes in inflammatory response. (A, C) Inducible nitric oxide synthase (iNOS) (131 kDa) and microsomal prostaglandin E synthase-1 (mPGES-1) (17 kDa) in stimulated RAW 264 cells. Lane 1, control (stimulated); Lane 2, AMLE (1.0 mg/mL). The experiments were performed in triplicate, and a representative result is presented. (B, D) Relative band densities of iNOS and mPGES-1 normalized to β-actin (42 kDa). Values are presented as the mean±SD (n=3). Different letters (a, b) indicate a significant difference (P<0.05) between groups. RAW 264, mouse macrophage cell line; LPS, lipopolysaccharides.

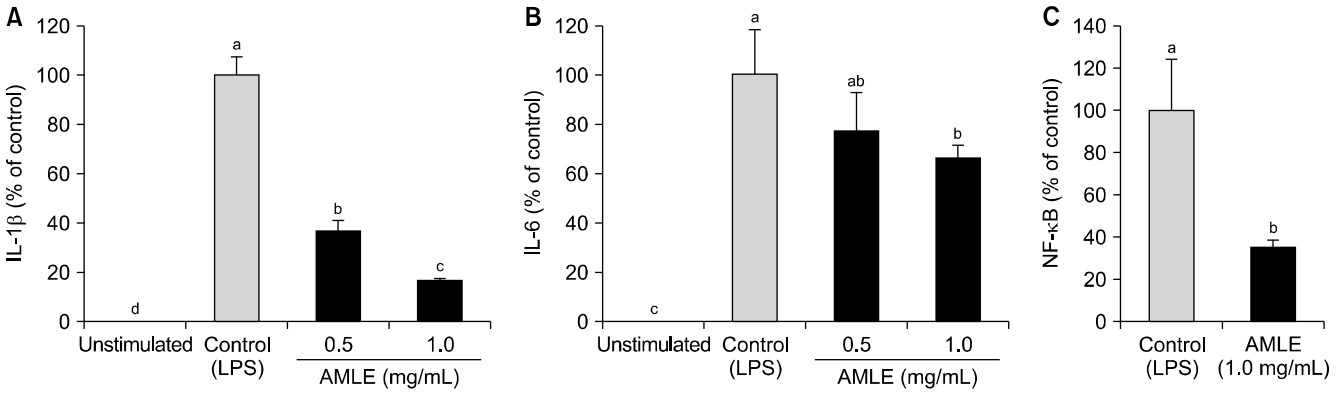

Fig. 7. Effects of *Astragalus membranaceus* leaf extract (AMLE) on cytokine release and nuclear translocation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) in inflammatory response. (A, B) Interleukin (IL)-1β and IL-6 release from stimulated RAW 264 cells. (C) Relative NF-κB levels in the nuclear fraction of stimulated RAW 264 cells. Values are presented as the mean±SD (n=3). Different letters (a-d) indicate significant differences (P<0.05) between groups. RAW 264, mouse macrophage cell line; LPS, lipopolysaccharides.

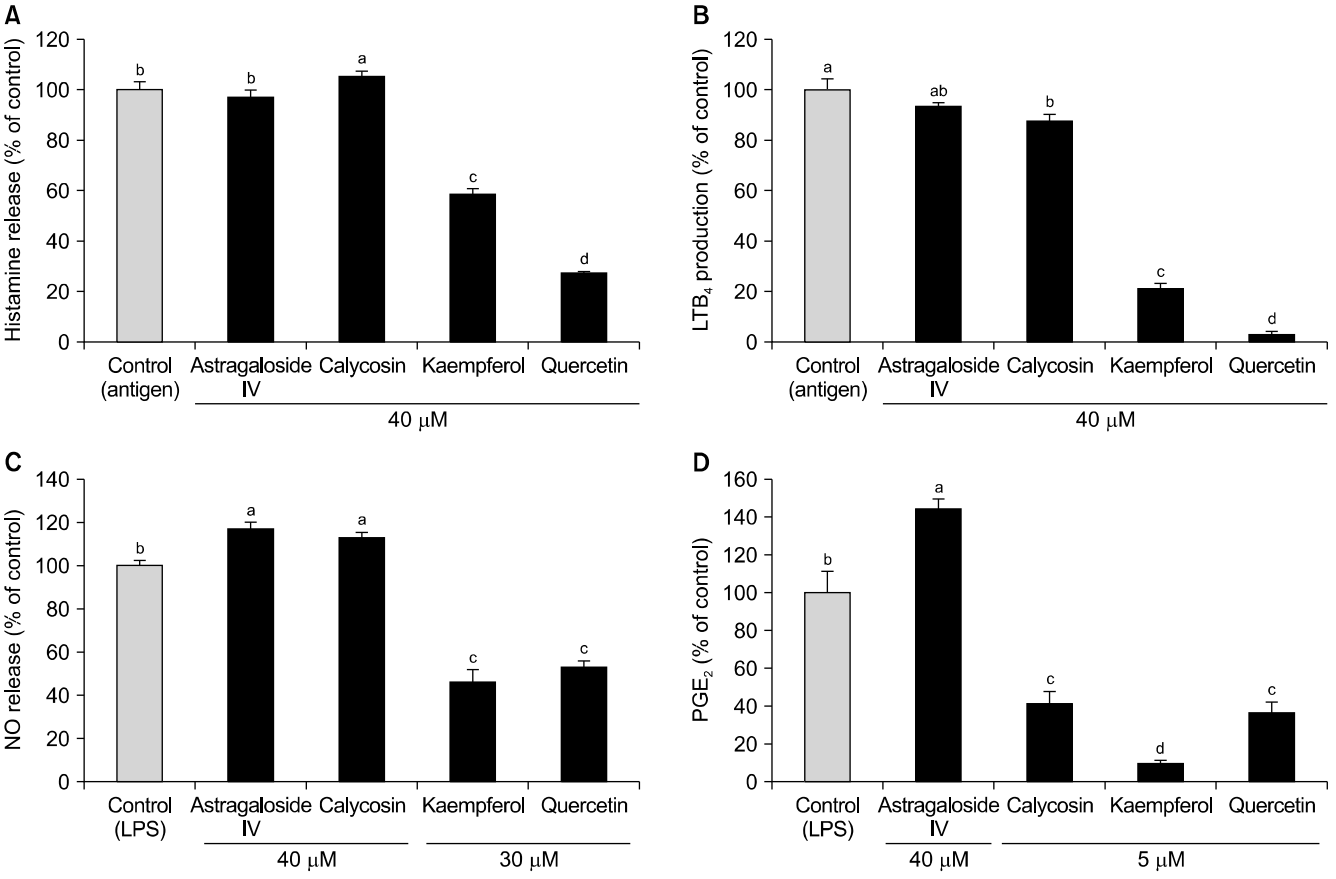

Fig. 8. Effects of major *Astragalus membranaceus* leaf compounds on the release of chemical mediators in allergic and inflammatory cell responses. (A) Release of histamine from stimulated RBL-2H3 cells. (B) Production of leukotrienes (LT) B_4 by stimulated PB-3c cells. (C) Nitric oxide (NO) release from stimulated RAW 264 cells. (D) Prostaglandin (PG) E_2 release from stimulated RAW 264 cells. Values are presented as the mean±SD (n=3). Different letters (a-d) indicate significant differences (P<0.05) between groups. RBL-2H3, rat basophilic leukemia cell line; PB-3c, mouse mast cell line; RAW 264, mouse macrophage cell line; LPS, lipopolysaccharides.

and quercetin suppressed the production of LTB_4 in stimulated PB-3c cells at 40 μM, with the latter showing a more potent inhibition than the former. Moreover, kaempferol and quercetin significantly inhibited NO release from stimulated RAW 264 cells at 30 μM, with no considerable difference between their activities. Calycosin did not inhibit NO release at 40 μM. Calycosin, kaempferol, and quercetin significantly suppressed PGE_2 release from stimulated RAW 264 cells at 5 μM. Among them, kaempferol exhibited the strongest inhibitory ef-

fect, surpassing those of calycosin and quercetin. Meanwhile, calycosin and quercetin showed similar activities. The results of cell viability assay confirmed that astragaloside IV and calycosin were not cytotoxic to RBL-2H3, PB-3c, and RAW 264 cells at concentrations up to 40 μM (Supplementary Fig. 2). Similarly, kaempferol and quercetin were nontoxic to RBL-2H3 and PB-3c cells at concentrations up to 40 μM and to RAW 264 cells at concentrations up to 30 μM (Supplementary Fig. 2). In AMLE, kaempferol (32.8±0.1 μmol/g dry weight) and quercetin (28.6±0.7 μmol/g dry weight) were the primary flavonol aglycones.

DISCUSSION

Natural plant-derived compounds, including polyphenols, are considered as promising alternatives to conventional Western medicine for mitigating the symptoms of allergic inflammation (Bellik et al., 2012). Using these compounds is expected to offer safe and effective treatment while minimizing the side effects that are commonly associated with conventional medicine. *A. membranaceus* roots have been used as a component of traditional Chinese herbal medicine. They contain astragaloside IV, a type of saponin with anticancer and neuroprotective effects (Liang et al., 2023). Conversely, owing to their general health benefits, *A. membranaceus* leaves have primarily been consumed as herbal teas and have not been explored as pharmaceutical agents. Furthermore, the specific bioactivities of *A. membranaceus* leaves remain unclear. The present study aimed to evaluate the inhibitory effects of AMLE on allergic and inflammatory responses in cell line models and to identify the bioactive compounds responsible for these effects.

Yamada et al. (1999) proposed that the antioxidant properties of phenolic compounds could be involved in suppressing the production of LTB_4 by mast cells. In addition, epigallocatechin gallate, a polyphenol predominantly found in green tea, exhibits potent antioxidant and antiallergic activities (Yamada and Tachibana, 2000). We previously demonstrated that food extracts containing polyphenols possess strong antiallergic and anti-inflammatory activities (Zorig et al., 2021; Qiu et al., 2023; Ochir et al., 2024). These findings suggest that polyphenols with antioxidant properties may exert antiallergic and anti-inflammatory effects. To confirm this, we determined the polyphenol contents of AMLE and AMRE and evaluated their radical scavenging abilities as measures of antioxidant activity. As shown in Fig. 1, AMLE contained a considerably higher amount of polyphenols (A) and exhibited a much stronger radical scavenging ability compared with AMRE (B). These findings indicate that the abundant polyphenols in AMLE contribute to its potent antioxidant activity.

Inhibiting the release of chemical mediators from basophils and mast cells is a critical mechanism for suppressing type I allergic responses (Duangmee et al., 2022). Many studies have evaluated the antiallergic activity of natural components by measuring the β-hexosaminidase activity in culture supernatants, which reflects the degranulation of basophilic leukocytes upon antigen stimulation. In the present study, the antiallergic effects of AMLE were assessed by directly measuring the release of histamine, a key chemical mediator responsible for allergic symptoms, from RBL-2H3 cells stimulated by antigen-IgE reactions (Matsuo et al., 1997; von Vietinghoff et al., 2006; Zorig et al., 2021). As shown in Fig. 2A and 2C, AMLE significantly inhibited the release of histamine from stimulated RBL-2H3 cells in a concentration-dependent manner, with AMLE tending to have a stronger activity than AMRE. In addition, LTB_4, another chemical mediator generated through the 5-LOX-mediated metabolism of arachidonic acid in activated mast cells, was analyzed. In a previous study, we established a method to evaluate the inhibitory effects of food components on the production of LTB_4 using the PB-3c mast cell line (Takasugi et al., 2018). This enzymatic reaction involves lipid peroxidation, which is inhibited by antioxidants (Santangelo et al., 2007; Schneider et al., 2007; Yahfoufi et al., 2018). As shown in Fig. 2B and 2D, AMLE significantly inhibited the production of LTB_4 in stimulated PB-3c cells in a concentration-dependent manner, with AMLE exhibit a stronger activity than AMRE. The results shown in Fig. 1 and 2 suggest that the polyphenols with antioxidant properties in AMLE may contribute to its ability to inhibit chemical mediators.

During mast cell activation, Ca^{2+} influx from the endoplasmic reticulum into the cytosol is a crucial step in intracellular signal transduction, which facilitates the release of histamine and production of LTB_4. Therefore, measuring the changes in intracellular Ca^{2+} concentrations is essential to elucidate the antiallergic mechanisms of pharmaceuticals and food-derived bioactive compounds (Zhang et al., 2023). As shown in Fig. 3A, AMLE inhibited the stimulation-induced increase in the cytosolic Ca^{2+} concentration in RBL-2H3 cells in a concentration-dependent manner. Once Ca^{2+} influx occurs in stimulated mast cells, the arachidonic acid cascade is induced via 5-LOX reaction (Siraganian, 2003). To elucidate the mechanism underlying the inhibitory effect of AMLE on the production of LTB_4 in stimulated PB-3c cells, we examined the effects of AMLE on 5-LOX mRNA expression following cell stimulation. As shown in Fig. 3, AMLE suppressed Ca^{2+} influx and 5-LOX expression after stimulation, indicating that they are key contributors to its inhibitory effects on chemical mediators. During intracellular signaling within stimulated mast cells, a phosphor-

ylation cascade of signaling molecules occurs upstream of Ca^{2+} influx (Gilfillan and Rivera, 2009). Future studies should examine the phosphorylation of signaling molecules to provide a more detailed mechanism underlying the inhibitory effects of AMLE on the release of chemical mediators.

The chemical mediators released by mast cells increase vascular permeability and promote the migration of other immune cells to the site of allergic reactions, subsequently inducing inflammatory responses wherein macrophages play a central role (Pal et al., 2020; Baran et al., 2023). Macrophages are activated when foreign substances (e.g., LPS) bind to TLRs on the cell membrane. This interaction triggers intracellular signaling pathways, which leads to the nuclear translocation of NF-κB and subsequent expression of inflammatory mediator synthases and inflammatory cytokines (Fatmawati et al., 2019; Pandya et al., 2022). We examined the effects of AMLE on LPS-stimulated inflammatory responses in macrophages to assess its anti-inflammatory effects. As shown in Fig. 4, AMLE significantly suppressed the release of the chemical mediators NO and PGE_2. Conversely, AMRE did not inhibit their release. Furthermore, AMLE significantly suppressed the mRNA (Fig. 5) and protein expression (Fig. 6B and 6D) of iNOS and mPGES-1, which are the key enzymes responsible for NO and PGE_2 synthesis, respectively, in stimulated macrophages. These results suggest that the inhibition of NO and PGE_2 release from LPS-stimulated RAW 264 cells by AMLE may result from the downregulation of these synthetic enzymes following stimulation. As shown in Fig. 7A and 7B, AMLE also significantly decreased the release of proinflammatory cytokines IL-1β and IL-6 in LPS-stimulated RAW 264 cells. Upon activation of macrophage intracellular signaling via TLR-mediated stimulation, the inhibitor of kappa B (IκB), which forms a complex with NF-κB, is degraded through phosphorylation and subsequent ubiquitination (Zhang et al., 2001; Israël, 2010). The freed NF-κB translocates into the nucleus, binds to the promoter regions of inflammation-related genes, and enhances their transcription, producing proteins that drive inflammatory processes. As shown in Fig. 7C, AMLE inhibited the nuclear translocation of NF-κB in stimulated macrophages. This finding suggests that the ability of AMLE to suppress inflammatory mediators and proinflammatory cytokines may be attributed to the inhibition of intracellular signaling pathways in macrophages. However, further research is needed to evaluate the effects of AMLE on IκB phosphorylation and ubiquitination to elucidate the specific mechanisms underlying its modulation of the NF-κB signaling pathway.

A. membranaceus leaves contain astragaloside IV and polyphenols, among which calycosin, kaempferol, quercetin, and their glycosides have the highest concentrations (Fu et al., 2014; Li et al., 2019). As described previously, AMLE is rich in polyphenols with antioxidant properties, potentially contributing to its inhibitory effects on allergic and inflammatory responses. To determine the active compounds that are responsible for these effects, we assessed the inhibitory activities of astragaloside IV, calycosin, kaempferol, and quercetin on the release of chemical mediators during allergic and inflammatory responses. As shown in Fig. 8, astragaloside IV showed no significant inhibitory effects on the release of chemical mediators from the stimulated immune cells in either the allergic or inflammatory models at 40 μM. Calycosin primarily inhibited PGE_2 release in LPS-stimulated RAW 264 cells. By contrast, kaempferol and quercetin exerted broad inhibitory effects, which significantly suppressed the release of all chemical mediators examined in allergic and inflammatory responses. Among them, quercetin displayed particularly strong inhibitory activity. Previous studies have reported the antiallergic properties of kaempferol (Lee et al., 2010) and quercetin (Mlcek et al., 2016; Zhang et al., 2023) and the anti-inflammatory activities of calycosin glycoside (Dong et al., 2018), kaempferol (Palacz-Wrobel et al., 2017; Wang et al., 2018; Li et al., 2023b), and quercetin (Endale et al., 2013; Wang et al., 2015), which are consistent with our findings. The results of quantitative analysis revealed that AMLE contains approximately 30 μmol/g of kaempferol and quercetin in aglycone equivalents. An effective concentration of 1 mg/mL of AMLE, which inhibits the release of chemical mediators in allergic and inflammatory responses, corresponds to approximately 30 μM of kaempferol and quercetin, which have been reported to possess significant antioxidant activity (Xu et al., 2019; Tian et al., 2021). These results suggest that the antiallergic and anti-inflammatory properties of AMLE are largely attributed to the combined effects of its major polyphenols, particularly kaempferol and quercetin, which exhibit strong antioxidant activities. Further research is needed to elucidate the mechanisms underlying the antiallergic and anti-inflammatory effects of these polyphenols in AMLE.

In conclusion, we found that AMLE effectively suppressed allergic and inflammatory responses. In particular, kaempferol and quercetin, which are the predominant polyphenols with antioxidant properties in *A. membranaceus* leaves, may play central roles by inhibiting intracellular signal transduction in immune cells. Based on these findings, *A. membranaceus* leaves could be used as a functional food for mitigating allergic and inflammatory symptoms. Future studies should use in vitro cell line models to elucidate the underlying mechanisms of AMLE. The relatively high concentrations of AMLE and its polyphenols used in this study reflect the experimental conditions under which immune cells were strongly stimu-

lated, necessitating higher doses to achieve significant suppression of the response. To address this issue, in vivo studies using animal models are needed to evaluate the efficacy and safety of AMLE at lower doses. Such studies should also consider physiological factors, including digestion, absorption, biodistribution, and metabolism, to provide a more comprehensive understanding of the therapeutic potential of AMLE.

FUNDING

None.

AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Concept and design: HA. Analysis and interpretation: all authors. Data collection: PB, MN, AZ. Writing the article: PB, HA, MT. Critical revision of the article: HA, MT, CHF. Final approval of the article: all authors. Statistical analysis: PB, MT. Overall responsibility: HA.

SUPPLEMENTARY MATERIALS

Supplementary materials can be found via https://doi.org/10.3746/pnf.2025.30.1.68

REFERENCES

Adesso S, Russo R, Quaroni A, Autore G, Marzocco S. *Astragalus membranaceus* extract attenuates inflammation and oxidative stress in intestinal epithelial cells via NF-κB activation and Nrf2 response. Int J Mol Sci. 2018. 19:800. https://doi.org/10.3390/ijms19030800

Almatroudi A, Mousa AM, Vinnakota D, Abalkhail A, Alwashmi ASS, Almatroodi SA, et al. Prevalence and associated factors of respiratory allergies in the Kingdom of Saudi Arabia: A cross-sectional investigation, September-December 2020. PLoS One. 2021. 16:e0253558. https://doi.org/10.1371/journal.pone.0253558

Ashina K, Tsubosaka Y, Nakamura T, Omori K, Kobayashi K, Hori M, et al. Histamine induces vascular hyperpermeability by increasing blood flow and endothelial barrier disruption *in vivo*. PLoS One. 2015. 10:e0132367. https://doi.org/10.1371/journal.pone.0132367

Baran J, Sobiepanek A, Mazurkiewicz-Pisarek A, Rogalska M, Gryciuk A, Kuryk L, et al. Mast cells as a target—A comprehensive review of recent therapeutic approaches. Cells. 2023. 12:1187. https://doi.org/10.3390/cells12081187

Bellik Y, Boukraâ L, Alzahrani HA, Bakhotmah BA, Abdellah F, Hammoudi SM, et al. Molecular mechanism underlying anti-inflammatory and anti-allergic activities of phytochemicals: An update. Molecules. 2012. 18:322-353. https://doi.org/10.3390/molecules18010322

Björk J, Hedqvist P, Arfors KE. Increase in vascular permeability induced by leukotriene B_4 and the role of polymorphonuclear leukocytes. Inflammation. 1982. 6:189-200. https://doi.org/10.1007/bf00916243

Castell M, Pérez-Cano FJ, Abril-Gil M, Franch À. Flavonoids on allergy. Curr Pharm Des. 2014. 20:973-987. https://doi.org/10.2174/13816128113199990041

Chapin RM. The determination of cresol by the phenol reagent of Folin and Denis. J Biol Chem. 1921. 47:309-314.

Chen XY, Han HF, He ZY, Xu XG. Immune mechanism, gene module, and molecular subtype identification of astragalus membranaceus in the treatment of dilated cardiomyopathy: An integrated bioinformatics study. Evid Based Complement Alternat Med. 2021. 2021:2252832. https://doi.org/10.1155/2021/2252832

Chin YW, Balunas MJ, Chai HB, Kinghorn AD. Drug discovery from natural sources. AAPS J. 2006. 8:E239-E253. https://doi.org/10.1007/bf02854894

D'Avino D, Cerqua I, Ullah H, Spinelli M, Di Matteo R, Granato E, et al. Beneficial effects of *Astragalus membranaceus* (Fisch.) bunge extract in controlling inflammatory response and preventing asthma features. Int J Mol Sci. 2023. 24:10954. https://doi.org/10.3390/ijms241310954

Dong L, Yin L, Chen R, Zhang Y, Hua S, Quan H, et al. Anti-inflammatory effect of Calycosin glycoside on lipopolysaccharide-induced inflammatory responses in RAW 264.7 cells. Gene. 2018. 675:94-101. https://doi.org/10.1016/j.gene.2018.06.057

Duangmee K, Boonmuang P, Santimaleeworagun W, Prasitdumrong H. Urticaria, angioedema, and type I hypersensitivity reactions associated with fibrinolytic agents. Asian Pac J Allergy Immunol. 2022. 40:379-385. https://doi.org/10.12932/ap-050619-0574

Endale M, Park SC, Kim S, Kim SH, Yang Y, Cho JY, et al. Quercetin disrupts tyrosine-phosphorylated phosphatidylinositol 3-kinase and myeloid differentiation factor-88 association, and inhibits MAPK/AP-1 and IKK/NF-κB-induced inflammatory mediators production in RAW 264.7 cells. Immunobiology. 2013. 218:1452-1467. https://doi.org/10.1016/j.imbio.2013.04.019

Fatmawati F, Erizka E, Hidayat R. Royal jelly (bee product) decreases inflammatory response in Wistar rats induced with ultraviolet radiation. Open Access Maced J Med Sci. 2019. 7:2723-2727. https://doi.org/10.3889/oamjms.2019.704

Fu J, Wang Z, Huang L, Zheng S, Wang D, Chen S, et al. Review of the botanical characteristics, phytochemistry, and pharmacology of *Astragalus membranaceus* (Huangqi). Phytother Res. 2014. 28:1275-1283. https://doi.org/10.1002/ptr.5188

Gilfillan AM, Rivera J. The tyrosine kinase network regulating mast cell activation. Immunol Rev. 2009. 228:149-169. https://doi.org/10.1111/j.1600-065X.2008.00742.x

Israël A. The IKK complex, a central regulator of NF-κB activation. Cold Spring Harb Perspect Biol. 2010. 2:a000158. https://doi.org/10.1101/cshperspect.a000158

Kawai M, Hirano T, Higa S, Arimitsu J, Maruta M, Kuwahara Y, et al. Flavonoids and related compounds as anti-allergic substances. Allergol Int. 2007. 56:113-123. https://doi.org/10.2332/allergolint.R-06-135

Kawasaki M, Toyoda M, Teshima R, Sawada J, Hayashi T, Arisawa M, et al. In vitro antiallergic activity of flavonoids in histamine release assay using rat basophilic leukemia (RBL-2H3) cells. J Food Hyg Soc Jpn. 1994. 35:497-503.

Kumar S, Pandey AK. Chemistry and biological activities of

flavonoids: An overview. Sci World J. 2013. 2013:162750. https://doi.org/10.1155/2013/162750

Ko EY, Nile SH, Jung YS, Keum YS. Antioxidant and antiplatelet potential of different methanol fractions and flavonols extracted from onion (*Allium cepa* L.). 3 Biotech. 2018. 8:155. https://doi.org/10.1007/s13205-018-1184-4

Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature. 1970. 227:680-685. https://doi.org/10.1038/227680a0

Lee EJ, Ji GE, Sung MK. Quercetin and kaempferol suppress immunoglobulin E-mediated allergic inflammation in RBL-2H3 and Caco-2 cells. Inflamm Res. 2010. 59:847-854. https://doi.org/10.1007/s00011-010-0196-2

Lee HN, Shin SA, Choo GS, Kim HJ, Park YS, Kim BS, et al. Anti-inflammatory effect of quercetin and galangin in LPS-stimulated RAW264.7 macrophages and DNCB-induced atopic dermatitis animal models. Int J Mol Med. 2018. 41:888-898. https://doi.org/10.3892/ijmm.2017.3296

Lee WS, Shin JS, Jang DS, Lee KT. Cnidilide, an alkylphthalide isolated from the roots of *Cnidium officinale*, suppresses LPS-induced NO, PGE_2, IL-1β, IL-6 and TNF-α production by AP-1 and NF-κB inactivation in RAW 264.7 macrophages. Int Immunopharmacol. 2016. 40:146-155. https://doi.org/10.1016/j.intimp.2016.08.021

Li J, Chen J, Huang P, Cai Z, Zhang N, Wang Y, et al. The anti-inflammatory mechanism of flaxseed linusorbs on lipopolysaccharide-induced RAW 264.7 macrophages by modulating TLR4/NF-κB/MAPK pathway. Foods. 2023a. 12:2398. https://doi.org/10.3390/foods12122398

Li K, Yu XH, Maskey AR, Musa I, Wang ZZ, Garcia V, et al. Cytochrome P450 3A4 suppression by epimedium and active compound kaempferol leads to synergistic anti-inflammatory effect with corticosteroid. Front Pharmacol. 2023b. 13:1042756. https://doi.org/10.3389/fphar.2022.1042756

Li Y, Guo S, Zhu Y, Yan H, Qian DW, Wang HQ, et al. Comparative analysis of twenty-five compounds in different parts of *Astragalus membranaceus* var. *mongholicus* and *Astragalus membranaceus* by UPLC-MS/MS. J Pharm Anal. 2019. 9:392-399. https://doi.org/10.1016/j.jpha.2019.06.002

Liang Y, Chen B, Liang D, Quan X, Gu R, Meng Z, et al. Pharmacological effects of astragaloside IV: A review. Molecules. 2023. 28:6118. https://doi.org/10.3390/molecules28166118

Liu T, Zhang L, Joo D, Sun SC. NF-κB signaling in inflammation. Signal Transduct Target Ther. 2017. 2:17023. https://doi.org/10.1038/sigtrans.2017.23

Lotfi R, Davoodi A, Mortazavi SH, Gorgin Karaji A, Tarokhian H, Rezaiemanesh A, et al. Imbalanced serum levels of resolvin E1 (RvE1) and leukotriene B4 (LTB4) in patients with allergic rhinitis. Mol Biol Rep. 2020. 47:7745-7754. https://doi.org/10.1007/s11033-020-05849-x

Matsuo N, Yamada K, Shoji K, Mori M, Sugano M. Effect of tea polyphenols on histamine release from rat basophilic leukemia (RBL-2H3) cells: the structure-inhibitory activity relationship. Allergy. 1997. 52:58-64. https://doi.org/10.1111/j.1398-9995.1997.tb02546.x

Mlcek J, Jurikova T, Skrovankova S, Sochor J. Quercetin and its anti-allergic immune response. Molecules. 2016. 21:623. https://doi.org/10.3390/molecules21050623

Nicoletti M, Maccauro G, Tripodi D, Saggini A, Potalivo G, Castellani ML, et al. Impact of IL-33 on PGD2 generation by activated human cord blood-derived mast cell: lack of effect on tryptase release. Eur J Inflamm. 2012. 10:473-482. https://doi.org/10.1177/1721727X1201000323

Ochir S, Zorig A, Wang Q, Takasugi M, Yamagishi T, Arai H. Isolation of two flavonol glycosides from *Rosa rugosa* pollen and their anti-inflammatory effect in lipopolysaccharide-stimulated macrophages. Food Sci Technol Res. 2024. 30:431-438. https://doi.org/10.3136/fstr.fstr-d-23-00178

Oh YC, Kang OH, Choi JG, Lee YS, Brice OO, Jung HJ, et al. Anti-allergic activity of a platycodon root ethanol extract. Int J Mol Sci. 2010. 11:2746-2758. https://doi.org/10.3390/ijms11072746

Pal S, Nath S, Meininger CJ, Gashev AA. Emerging roles of mast cells in the regulation of lymphatic immuno-physiology. Front Immunol. 2020. 11:1234. https://doi.org/10.3389/fimmu.2020.01234

Palacz-Wrobel M, Borkowska P, Paul-Samojedny M, Kowalczyk M, Fila-Danilow A, Suchanek-Raif R, et al. Effect of apigenin, kaempferol and resveratrol on the gene expression and protein secretion of tumor necrosis factor alpha (TNF-α) and interleukin-10 (IL-10) in RAW-264.7 macrophages. Biomed Pharmacother. 2017. 93:1205-1212. https://doi.org/10.1016/j.biopha.2017.07.054

Pandya P, Kublo L, Stewart-Ornstein J. p53 promotes cytokine expression in melanoma to regulate drug resistance and migration. Cells. 2022. 11:405. https://doi.org/10.3390/cells11030405

Qiu S, Zorig A, Sato N, Yanagihara A, Kanazawa T, Takasugi M, et al. Effect of polyphenols in sea buckthorn berry on chemical mediator release from mast cells. Prev Nutr Food Sci. 2023. 28:335-346. https://doi.org/10.3746/pnf.2023.28.3.335

Santangelo C, Varì R, Scazzocchio B, Di Benedetto R, Filesi C, Masella R. Polyphenols, intracellular signalling and inflammation. Ann Ist Super Sanita. 2007. 43:394-405.

Schneider C, Pratt DA, Porter NA, Brash AR. Control of oxygenation in lipoxygenase and cyclooxygenase catalysis. Chem Biol. 2007. 14:473-488. https://doi.org/10.1016/j.chembiol.2007.04.007

Siraganian RP. Mast cell signal transduction from the high-affinity IgE receptor. Curr Opin Immunol. 2003. 15:639-646. https://doi.org/10.1016/j.coi.2003.09.010

Song Y, Wang M, Xie J, Li W, Zhang X, Wang T, et al. Prevalence of allergic rhinitis among elementary and middle school students in Changsha city and its impact on quality of life. J Laryngol Otol. 2015. 129:1108-1114. https://doi.org/10.1017/s0022215115002492

Takasugi M, Muta E, Yamada K, Arai H. A new method to evaluate anti-allergic effect of food component by measuring leukotriene B_4 from a mouse mast cell line. Cytotechnology. 2018. 70:177-184. https://doi.org/10.1007/s10616-017-0129-9

Tan X, Xu M, Liu F, Xu M, Yao Y, Tang D. Antimetastasis effect of *Astragalus membranaceus-Curcuma zedoaria* via β-catenin mediated CXCR4 and EMT signaling pathway in HCT116. Evid Based Complement Alternat Med. 2019. 2019:9692350. https://doi.org/10.1155/2019/9692350

Tian C, Liu X, Chang Y, Wang R, Lv T, Cui C, et al. Investigation of the anti-inflammatory and antioxidant activities of luteolin, kaempferol, apigenin and quercetin. S Afr J Bot. 2021. 137:257-264. https://doi.org/10.1016/j.sajb.2020.10.022

von Vietinghoff V, Gäbel G, Aschenbach JR. High-performance liquid chromatographic method for the determination of histamine and 1-methylhistamine in biological buffers. J Chromatogr B Analyt Technol Biomed Life Sci. 2006. 844:335-339. https://doi.org/10.1016/j.jchromb.2006.07.017

Verghese A, Arora JS, Sharma U, Rai A, Chattopadhya D. Divergent pro-inflammatory cytokine response induced by GB virus C/ hepatitis G virus (GBV-C/HGV) and Torque Teno virus (TTV) co-infections in hepatitis C virus (HCV) infected thalassemia patients. J Mol Biol Res. 2018. 8:137-152. https://doi.org/10.5539/jmbr.v8n1p137

Wang B, Wu L, Chen J, Dong L, Chen C, Wen Z, et al. Metabolism pathways of arachidonic acids: mechanisms and potential therapeutic targets. Signal Transduct Target Ther. 2021. 6:94. https://doi.org/10.1038/s41392-020-00443-w

Wang J, Fang X, Ge L, Cao F, Zhao L, Wang Z, et al. Antitumor, antioxidant and anti-inflammatory activities of kaempferol and its corresponding glycosides and the enzymatic preparation of kaempferol. PLoS One. 2018. 13:e0197563. https://doi.org/10.1371/journal.pone.0197563

Wang ZB, Zhai YD, Ma ZP, Yang CJ, Pan R, Yu JL, et al. Triterpenoids and flavonoids from the leaves of *Astragalus membranaceus* and their inhibitory effects on nitric oxide production. Chem Biodivers. 2015. 12:1575-1584. https://doi.org/10.1002/cbdv.201400371

Xu D, Hu MJ, Wang YQ, Cui YL. Antioxidant activities of quercetin and its complexes for medicinal application. Molecules. 2019. 24:1123. https://doi.org/10.3390/molecules24061123

Yagi S, Furukawa S, Suzuki S, Ohashi K, Tomida H, Yamamoto Y, et al. No association between allergic diseases and constipation in Japanese ulcerative colitis patients: A cross-sectional study. Cureus. 2024. 16:e55912. https://doi.org/10.7759/cureus.55912

Yahfoufi N, Alsadi N, Jambi M, Matar C. The immunomodulatory and anti-inflammatory role of polyphenols. Nutrients. 2018. 10:1618. https://doi.org/10.3390/nu10111618

Yamada K, Shoji K, Mori M, Ueyama T, Matsuo N, Oka S, et al. Structure-activity relationship of polyphenols on inhibition of chemical mediator release from rat peritoneal exudate cells. In Vitro Cell Dev Biol Anim. 1999. 35:169-174. https://doi.org/10.1007/s11626-999-0020-x

Yamada K, Tachibana H. Recent topics in anti-oxidative factors. Biofactors. 2000. 13:167-172. https://doi.org/10.1002/biof.5520130127

Zhao G, Zhang T, Ma X, Jiang K, Wu H, Qiu C, et al. Oridonin attenuates the release of pro-inflammatory cytokines in lipopolysaccharide-induced RAW264.7 cells and acute lung injury. Oncotarget. 2017. 8:68153-68164. https://doi.org/10.18632/oncotarget.19249

Zhang A, Sun H, Wang X. Recent advances in natural products from plants for treatment of liver diseases. Eur J Med Chem. 2013. 63:570-577. https://doi.org/10.1016/j.ejmech.2012.12.062

Zhang G, Ghosh S. Toll-like receptor-mediated NF-κB activation: a phylogenetically conserved paradigm in innate immunity. J Clin Invest. 2001. 107:13-19. https://doi.org/10.1172/jci11837

Zhang Y, Guan R, Huang H. Anti-allergic effects of quercetin and quercetin liposomes in RBL-2H3 cells. Endocr Metab Immune Disord Drug Targets. 2023. 23:692-701. https://doi.org/10.2174/1871530322666220627151830

Zorig A, Toko R, Sukhbold E, Takasugi M, Arai H. *Echinacea purpurea* water extracts suppress the release of chemical mediators from mast cells. Biosci Biotechnol Biochem. 2021. 85:931-940. https://doi.org/10.1093/bbb/zbaa125