

# Ameliorative effects of *Paulownia tomentosa* flower absolute on atopic dermatitis-related responses in mast cells and keratinocytes and its chemical composition

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**Abstract.** *Paulownia tomentosa* (PT) exhibits diverse biological activities, including anti-inflammatory and antioxidant effects; however, its potential effects on atopic dermatitis (AD) are unclear. The present study examined the effects of PT flower absolute (PTFAb) on AD-related responses, particularly mast cell activation and keratinocyte barrier-related responses. PTFAb was isolated from PT flowers using a solvent extraction method and its chemical composition was analyzed by gas chromatography/mass spectrometry, through which 13 components were identified. *In vitro* biological tests on rat basophilic leukemia cells (RBL-2H3 cells; a commonly used mast cell model) and human epidermal keratinocytes (HaCaT cells) were performed using water-soluble tetrazolium salt, 5-bromo-2'-deoxyuridine incorporation, Boyden chamber, immunoblotting and enzyme-linked immunosorbent assay methods. All experiments were conducted on cells at nontoxic PTFAb concentrations. PTFAb reduced the expression of the v-soluble N-ethylmaleimide-sensitive factor attachment protein receptor proteins vesicle-associated membrane protein (VAMP)7 and VAMP8 in RBL-2H3 cells. In RBL-2H3 cells stimulated with anti-dinitrophenyl (DNP)-immunoglobulin E and DNP-bovine serum albumin, PTFAb suppressed the release of  $\beta$ -hexosaminidase and histamine (indicators of mast cell degranulation), and the phosphorylation of spleen tyrosine kinase, phosphatidylinositol 3-kinase and protein kinase B.

PTFAb also induced proliferation, migration, and type I and IV collagen synthesis. Furthermore, PTFAb upregulated tumor necrosis factor- $\alpha$  (TNF- $\alpha$ )-reduced filaggrin expression and the expression of hyaluronan synthase (HAS)-2 and HAS-3 in HaCaT cells, and reduced TNF- $\alpha$ -increased intercellular adhesion molecule-1 expression in HaCaT cells. In conclusion, PTFAb may exert inhibitory effects on mast cell degranulation-related responses and potentiate skin barrier-related responses, suggesting its potential to modulate AD-related cellular responses or skin barrier function.

## Introduction

Atopic dermatitis (AD) is a representative chronic inflammatory skin disease caused by a combination of immune dysfunction and impaired skin barrier function. Its prevalence is steadily increasing worldwide (1). The pathogenesis of AD is shaped by a complex interplay of genetic predisposition, environmental stimuli, immunological abnormalities, and impaired skin barrier function (1). In particular, the excessive activation of the T helper type 2 (Th2) cell immune response acts as a key factor in exacerbating the inflammatory response of AD by promoting the overproduction of immunoglobulin E (IgE) antibodies in B cells and the sensitization and activation of mast cells (2). The binding of IgE and its receptors [high affinity IgE receptors (Fc $\epsilon$ RI)] activates mast cells and triggers mast cell degranulation, releasing various allergic or inflammatory mediators stored within the cells, such as cytokines, histamine, and  $\beta$ -hexosaminidase, contributing directly to the induction of itching and inflammatory skin lesions (2,3). Mast cell degranulation is regulated by membrane fusion between intracellular vesicles and the cell membrane. In addition, SNARE (soluble N-ethylmaleimide-sensitive factor attachment protein receptor) protein complexes play a key role in this process (4). In particular, vesicle membrane-located v(vesicle)-SNARE proteins, such as vesicle-associated membrane protein (VAMP)7 and VAMP8, bind to membrane-located t(target)-SNARE proteins, synaptosome-associated protein (SNAP) 23, and syntaxin family proteins, forming a quadruple-helix SNARE complex (4).

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The bound SNARE complex induces membrane fusion and degranulation, subsequently releasing allergic and inflammatory mediators from the cells (4). This degranulation response in activated mast cells is closely linked to the activation of spleen tyrosine kinase (Syk) and its downstream molecules, such as phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT) and mitogen-activated protein kinases (MAPKs) signaling pathways (5,6). Therefore, SNARE protein complexes and related signaling molecules are becoming important molecular targets for modulating mast cell-mediated allergic and inflammatory responses.

In addition to these immunological abnormalities, structural impairment of the skin barrier function is another key pathogenic factor determining the onset and exacerbation of AD (7). The normal skin barrier maintains homeostasis through the harmonious interaction of keratinocyte differentiation-linked structural proteins [filaggrin (FLG), loricrin (LOR), and involucrin (IVL)], a ceramide-centered lipid layer, and extracellular matrix (ECM) components (e.g., collagen), protecting the body from external harmful environments (8,9). FLG, LOR, and IVL are key structural proteins of the outer layer of the epidermis (stratum corneum), which is formed through the terminal differentiation of keratinocytes and is essential for the skin barrier function (7). These proteins regulate the skin barrier integrity and hydration, and deficiencies in them contribute to epidermal barrier dysfunction, a central feature of AD (7). In addition to structural proteins, proper hydration of the stratum corneum is critical for barrier homeostasis. Hyaluronan (also called hyaluronic acid) maintains skin moisture through its high water-binding capacity and is synthesized by hyaluronan synthase (HAS) isoforms (HAS-1, -2, and -3) (10,11). Alterations in hyaluronic acid synthesis and degradation have been linked to the development and progression of AD (11).

When the skin barrier is continuously damaged, the tissue promotes the proliferation and migration of keratinocytes and induces collagen synthesis, a key component of the ECM, to re-epithelialize and structurally repair the damaged area (12,13). On the other hand, the persistent inflammatory environment in AD disrupts this normal repair process, and incomplete tissue regeneration further exacerbates the barrier vulnerability (14). In a cytokine-rich inflammatory environment characteristic of AD, keratinocytes can show enhanced intercellular adhesion molecule-1 (ICAM-1) expression in response to inflammatory stimuli (15). This increased ICAM-1 expression may promote the infiltration of immune cells into the skin and enhance their interaction with ICAM-1, thereby triggering localized inflammatory processes (16). Therefore, controlling ICAM-1 expression regulation may help treat skin inflammatory diseases such as AD.

The adverse effects of long-term use of synthetic steroids and immunosuppressants have increased interest in safe and effective natural ingredients that can modulate the immune responses and restore the skin barrier (17). Plant essential oils contain a variety of secondary metabolites with the potential to exert diverse biological effects, including antibacterial, anti-inflammatory, and wound healing properties (18,19). Among these, *Paulownia tomentosa* (also called *Paulownia coreana* Uyeki at the National Institute of Biological Resources in Korea) is a deciduous tree belonging

to the Paulowniaceae family, native to Korea, China, and Japan (20). This plant is known for its timber and ornamental value and is used widely in construction, furniture making, musical instruments, and handicrafts (21). Traditionally, it has been used to treat bronchial diseases such as cough, asthma, and bronchitis (22). In addition, pharmacological studies have reported that extracts and bioactive compounds derived from various parts of the plant, including the flowers, leaves, and fruit, have diverse biological activities, such as anti-inflammatory, antioxidant, neuroprotective, and nitric oxide inhibitory effects (20,23,24).

Nevertheless, little research has been conducted on the effects of *Paulownia tomentosa* (PT) flower essential oil on mast cell degranulation and skin barrier function, which are related to the pathogenesis of AD. Although many topical and systemic treatments for AD have been developed (25), their clinical utility has been frequently limited because of their side effects and the complex etiology of the disease. Therefore, novel strategies are essential to overcome these existing limitations. In the present study, PT flower absolute (PTFAB) was isolated, and its chemical composition was analyzed by gas chromatography/mass spectrometry (GC/MS). The effects of PTFAB on AD-related pathological aspects were also investigated comprehensively, particularly mast cell degranulation, its modulatory signaling, and skin barrier function-related responses, *in vitro*. The following cells were used in this *in vitro* study: rat basophilic leukemia cells (RBL-2H3 cells) as a mast cell model and human keratinocytes (HaCaT cells) as an epithelial cell model.

## Materials and methods

**Materials.** Fetal bovine serum (FBS; cat. no. SH30919.03) and penicillin/streptomycin (P/S; cat. no. SH40003.01) were purchased from Hyclone (Logan, UT, USA). Trypsin-ethylenediaminetetraacetic acid (cat. no. 15400-054) was purchased from Gibco BRL (Gaithersburg, MD, USA). Phosphate-buffered saline (PBS; cat. no. LB 001-02) and Dulbecco's Modified Eagle Medium (DMEM; high glucose, cat. no. LM 001-05; low glucose, cat. no. LM 001-11) were supplied by Welgene (Daegu, Korea). Anti- $\beta$ -actin antibody (cat. no. A5441), anti-DNP IgE antibody (cat. no. D8406), *p*-nitrophenyl-*N*-acetyl- $\beta$ -D-glucosaminide (cat. no. N9376), Tween-20 (cat. no. P2287), sucrose (cat. no. S1888), polyvinylidene fluoride (PVDF) membrane (cat. no. IPVH00010), dimethyl sulfoxide (DMSO; cat. no. D2650), LY294002 (cat. no. 440202), SB203580 (cat. no. S8307), and PD98059 (cat. no. P215) were obtained from MilliporeSigma (St. Louis, MO, USA). Recombinant human keratinocyte growth factor (rhKGF; cat. no. 251-KG), recombinant human epidermal growth factor (rhEGF; cat. no. 236-EG) (purity >97%), Recombinant human tumor necrosis factor- $\alpha$  (rhTNF- $\alpha$ ; cat. no. 210-TA), and anti-ICAM-1 antibody (cat. no. BAF 796) were acquired from R&D Systems (Minneapolis, MN, USA). The EZ-CyTox kit (cat. no. EZ-3000) and bovine serum albumin (BSA; cat. No. A0100-010) were supplied by DoGenBio (Seoul, Korea) and GenDEPOT (Katy, TX, USA), respectively. Type I collagen (rat tail; cat. no. 354236) was sourced from Corning Life Sciences (Corning, NY, USA). The 5-bromo-2'-deoxyuridine (BrdU) kit (cat. no. 11669915001) and streptavidin-peroxidase

(POD) conjugate (cat. no. 11089153001) was obtained from Roche (Indianapolis, IN, USA). DNP-BSA (cat. no. A23018), monoclonal anti-type I and IV collagen antibodies (cat. no. MA1-26771 and cat. no. MA1-22148, respectively) and chemiluminescent substrate (cat. no. 37069) were obtained from Thermo Scientific (Waltham, MA, USA). The antibodies for phospho Syk (cat. no. 2711), ERK1/2 (cat. no. 9102), phospho ERK1/2 (cat. no. 9101), p38 MAPK (cat. no. 9212), phospho p38 MAPK (cat. no. 9211), JNK (cat. no. 9258), phospho JNK (cat. no. 4668), PI3K (cat. no. 4292), phospho PI3K (cat. no. 4228), AKT (cat. no. 9272), phospho AKT (cat. no. 9271), horseradish peroxidase conjugated rabbit immunoglobulin G (cat. no. 7074), and horseradish peroxidase conjugated mouse immunoglobulin G (cat. no. 7076) were purchased from Cell Signaling Technology, Inc. (Beverly, MA, USA). RIPA buffer (cat. no. 9806) was also purchased from the same company. The antibodies for Syk (cat. no. ab3993), VAMP7 (cat. no. ab36195), VAMP8 (cat. no. ab76021), Syntaxin 1a (cat. no. ab170890), Syntaxin 4 (cat. no. ab184545), SNAP 25 (cat. no. ab109105), and polyclonal anti-type I and IV collagens (cat. no. ab6577 and cat. no. ab6581, respectively) were obtained from Abcam (Cambridge, UK). The antibodies for SNAP 23 (cat. no. sc-166244) and filaggrin (cat. no. sc-66192) were supplied by Santa Cruz Biotechnology (Dallas, TX, USA). Anti-HAS-2 (cat. no. NBP2-37446) and anti-HAS-3 (cat. no. NBP1-86328) antibodies were purchased from Novus Biologicals (Littleton, CO, USA). n-Hexane (cat. no. 4081-4110) and ethanol (cat. no. 4204-4410) were obtained from DAEJUNG (Siheung, Korea) and piceatannol was from TCI (Tokyo, Japan).

**Extraction and preparation of *Paulownia tomentosa* flower absolute.** The flowers of *Paulownia tomentosa* were harvested in Daepyeong-ri, Gwangdeok-myeon, Cheonan, Korea (36.678200°N, 127.161120°E). Plant collection was conducted in accordance with the guidelines of the Korea Forest Service (Daejeon, Korea). *Paulownia tomentosa* is not classified as a protected or endangered species. Its conservation status is listed as 'Least Concern' in the IUCN Red List (26). It is classified not included in any protected or threatened category in national databases, including the Korean Red Data Book of Vascular Plants (27). The plant material was taxonomically authenticated by Dr. Hyun-Jun Kim at the Forest Medicinal Resources Research Center (Seoul, Korea). The voucher specimen (No. PCU-0001) has been deposited in publicly accessible herbaria at the Korea Essential Oil Resource Research Institute, Hoseo University (Asan, Korea), and in the Korea Forest Plants Essential Oil Bank of the National Institute of Forest Science (Seoul, Korea). The flower essential oil of *Paulownia tomentosa* was obtained as an absolute by organic solvent extraction. Briefly, 4 kg of *Paulownia tomentosa* flowers were subjected to extraction with n-hexane to obtain a concrete, which was then dissolved in ethanol. The solvent was evaporated, leaving 5 g of the absolute type of essential oil. The resulting absolute type of essential oil was a yellow, viscous liquid, with a final yield of 0.125% (w/w). The extracted absolute type of essential oil was stored at -80°C until further use. For subsequent cell-based experiments, the PTFab sample was dissolved in DMSO, to prepare a stock solution before treatment. The final concentration of DMSO was adjusted

to be identical in all experimental groups, including the PTFab-treated and control groups. For RBL-2H3 cell experiments, the final DMSO concentration was maintained at 0.1%, whereas for HaCaT cell experiments, it was maintained at 0.5%. The corresponding DMSO concentrations were used in the control groups as vehicle controls.

**Identification of compounds in PTFab.** PTFab analysis was conducted by NICEM (the National Instrumentation Center for Environmental Management, Seoul National University, Seoul, Korea). Its components were identified by GC/MS analysis using a TRACE 1310 GC unit coupled to an ISQ LT single-quadrupole mass spectrometer (Thermo Scientific, Waltham, MA, USA), as reported elsewhere (28). Briefly, the derivatized samples were subjected to separation on a DB-5MS column (60 m x 0.25 mm, 0.25 µm; Agilent Technologies, Santa Clara, CA, USA) at a constant flow rate of 1 ml/min using the following program: 50°C for 5 min, 50 to 65°C at 10°C/min, 65 to 210°C at 5°C/min, 210 to 310°C at 20°C/min, and 310°C for 10 min. The mass spectra were acquired in the range of m/z 35 to m/z 550 at 0.2 scans/sec. The transfer line and ion source temperatures were 300°C and 270°C, respectively. The detected compounds were identified by comparing the mass spectra and retention indices (RIs) with reference standards in the NIST/NIH/EPA mass spectral library (NIST 11, version 2.0 g) and by matching the retention times and spectra with those of commercially available standards. A solution of C<sub>7</sub>-C<sub>30</sub> n-alkanes was used as the standard to calculate the RIs.

**Cell culture.** RBL-2H3 cells (rat basophilic leukemia; cat. no. 22256), which were commonly used as a mast cell model (29,30), were obtained from the Korea Cell Line Bank (Seoul, Korea) and the HaCaT human keratinocyte cell line (Celosaurus CVCL\_0038) was obtained from the National Institute for Korean Medicine Development (Gyeongsan, Korea). According to the institute, the cells were originally sourced from Keimyung University (Daegu, Korea), which had purchased them from CLS Cell Lines Service (Cyton; cat. no. 300493, Eppelheim, Germany), the official DKFZ-approved supplier (31,32). Cells were maintained under standard culture conditions. Their identity and integrity were routinely confirmed by morphological assessment and monitoring of growth characteristics. All cultures were regularly tested for mycoplasma contamination, and cells between passages 28 and 35 were used in experiments. The cells were cultured in DMEM (Welgene Inc., Daegu, Korea) supplemented with 10% FBS (HyClone, Logan, UT, USA) and 1% P/S (HyClone) at 37°C in a humidified incubator containing 5% CO<sub>2</sub>.

**Cell viability assay.** The RBL-2H3 and HaCaT cells viability was assessed using a water-soluble tetrazolium salt (WST) assay with the EZ-CyTox kit (cat. no. EZ-3000; DoGenBio, Seoul, Korea). The cells were seeded at 5x10<sup>3</sup> cells per well in 96-well microtiter plates and incubated overnight in a humidified atmosphere containing 5% CO<sub>2</sub> and 95% air at 37°C. Cells were treated with various concentrations of PTFab for 24 h, while vehicle control cells received the corresponding concentration of DMSO. Finally, 10 µl of EZ-Cytox reagent (cat. no. EZ-3000; DoGenBio, Seoul, Korea) was added, and

the plate was incubated at 37°C for 30 min. The absorbance at 450 nm was determined using a multi-well plate reader (Synergy 2; BioTek Instruments, Winooski, VT, USA).

**Proliferation assay.** The HaCaT cell proliferation level was evaluated using a DNA synthesis-based BrdU incorporation assay with a BrdU kit (cat. no. 11669915001; Roche, Indianapolis, IN, USA). Briefly, 96-well black flat-bottom plates were coated with type I collagen (0.1 mg/ml), sourced from Corning Life Sciences (Corning, NY, USA), for 30 min, and HaCaT cells were seeded at  $2 \times 10^3$  cells/well and incubated for 12 h. The cells were treated with in PTFAb at the same concentrations described above, with rhEGF (50 ng/ml) as a positive control, and incubated for 36 h. Subsequently, BrdU labeling solution (1:200 dilution with serum-free medium) was added, and the cells were incubated for an additional 12 h at 37°C. After incubation, the cells were fixed at room temperature for 30 min and incubated with peroxidase-conjugated anti-BrdU antibody (1:100 dilution with diluent included in the BrdU kit) for 90 min at room temperature. After the substrate reaction, the luminescence was measured using a luminometer (Synergy 2; BioTek Instruments, USA).

**Migration assay.** The HaCaT cell migration was evaluated using a 48-well microchemotaxis chamber (cat. no. 866-417-0014; Neuro Probe, Gaithersburg, MD, USA). A polycarbonate membrane with an 8  $\mu$ m pore size (cat. no. 1220686; GVS Life Science, Bologna, Italy) and pre-coated with type I collagen (0.1 mg/ml) was used. The lower chambers were filled with DMEM containing 0.1% BSA, supplemented with rhEGF (1 ng/ml) as a positive control or PTFAb at various concentrations. After assembling the collagen-coated membrane and the upper chamber, the HaCaT cells were seeded into the upper chamber at a density of  $5 \times 10^4$  cells/well in 50  $\mu$ l of medium. The chamber was incubated at 37°C in a humidified atmosphere containing 5% CO<sub>2</sub> for 3 h and 30 min. After incubation, the membrane was removed from the chamber, and the migrated cells were fixed and stained using a Diff-Quick solution (cat. no. 38721; Sysmex Corporation, Kobe, Japan). The migrated cells were visualized under an optical microscope at x200 magnification, and their number was quantified by manual counting.

**Collagen synthesis assay.** HaCaT cells were seeded at a density of  $5 \times 10^5$  cells per 100-mm dish and incubated for 12 h at 37°C in a humidified atmosphere containing 5% CO<sub>2</sub>. After incubation, the culture medium was removed, and the cells were washed with PBS. The cells were then treated with PTFAb at the indicated concentrations and further incubated for 48 h under the same conditions. The conditioned medium (CM; supernatants) was collected and centrifuged at 500, 800, and 1,000  $\times$  g for 10 min each to obtain the supernatant. In parallel, the cells were washed with PBS, lysed using a lysis buffer, and the total protein concentration was determined using a DC protein assay kit (cat. no. 500-0116; Bio-Rad Laboratories, Hercules, CA, USA). For collagen quantification, 96-well black flat-bottom plates (cat. no. 655090; Greiner Bio-One, Kremsmünster, Austria) were coated with monoclonal antibodies against type I and type IV collagen (cat. no. MA1-26771 and cat. no. MA1-22148, respectively; Thermo Scientific,

Waltham, MA, USA) at 2  $\mu$ g/well in 100  $\mu$ l and incubated at 4°C for 12 h. After washing with PBS, the plates were blocked with PBS containing 1% BSA and 5% sucrose for 1 h at room temperature. The collected CM was then added to each well and incubated for 90 min at room temperature. After washing with PBS, biotinylated polyclonal antibodies against type I and type IV collagen (cat. no. ab6577 and cat. no. ab6581, respectively; Abcam, Cambridge, UK), diluted 1:2,000, were added and incubated for 90 min at room temperature. The wells were then washed and incubated with POD conjugate (Roche, Indianapolis, IN, USA) diluted 1:5,000 for 1 h. After a final wash with PBS, chemiluminescent substrate solution (Thermo Scientific) was added, and the luminescence was measured using a luminometer (Synergy 2; Bio-Tek Instruments).

**SNARE protein expression.** The RBL-2H3 cells were seeded at a density of  $3 \times 10^5$  cells per 100-mm culture dish and incubated at 37°C in a humidified atmosphere containing 5% CO<sub>2</sub> for 12 h. After incubation, the culture medium was removed, and the cells were washed with PBS. The cells were then treated with various concentrations of PTFAb and incubated at 37°C in an atmosphere containing 5% CO<sub>2</sub> for 48 h. The expression of SNARE proteins, including VAMP7, VAMP8, syntaxin 1a, syntaxin 4, SNAP 23 and SNAP 25, was analyzed by immunoblotting.

**$\beta$ -Hexosaminidase and histamine release assays.** The RBL-2H3 cells were seeded in 24-well plates at a density of  $1.5 \times 10^4$  cells per well and incubated at 37°C in a humidified atmosphere containing 5% CO<sub>2</sub> for 12 h. After incubation, the culture medium was removed, and the cells were washed with PBS. The cells were then treated with the indicated PTFAb and incubated for 48 h at 37°C with 5% CO<sub>2</sub>. Anti-DNP IgE (cat. no. D8406; MilliporeSigma, St. Louis, MO, USA) was then diluted in DMEM containing 10% FBS and added to the cells at a final concentration of 200 ng/ml, followed by incubation for 10 h at 37°C in a humidified atmosphere containing 5% CO<sub>2</sub>. After sensitization, the cells were washed with Siraganian buffer (cat. no. BS067; Biosolution, Seoul, Korea). Degranulation was induced by stimulation with DNP-BSA, diluted in Siraganian buffer to a final concentration of 20 ng/ml, and incubated in a humidified atmosphere containing 5% CO<sub>2</sub> for 1 h at 37°C. The culture supernatants were collected and centrifuged sequentially at 500, 1,000, and 10,000  $\times$  g for 10 min each to obtain CM. In parallel, cells were washed with PBS and lysed with a lysis buffer to extract the intracellular proteins, which were quantified using a DC protein assay.

For the  $\beta$ -hexosaminidase release assay, 50  $\mu$ l of the collected supernatant was mixed with 100  $\mu$ l of substrate buffer containing 2 mM 4-*p*-nitrophenyl-*N*-acetyl- $\beta$ -D-glucosaminide in 0.05 M sodium citrate buffer (pH 4.5) and incubated at 37°C for 3 h. The reaction was quenched by adding 100  $\mu$ l of a stop solution (0.2 M glycine-NaOH, pH 10.0), and the absorbance was measured at 405 nm using a multi-well plate reader (Synergy2; Bio-Tek Instruments).

Histamine release was quantified from the same conditioned medium using a histamine enzyme-linked immunoassay (EIA) kit (cat. no. A05890; Cayman Chemical, Ann Arbor, MI, USA), according to the manufacturer's instructions.

**Activation analysis of Syk, MAPKs, and PI3K/AKT.** The RBL-2H3 cells were seeded at a density of  $8 \times 10^5$  cells per 60-mm culture dish and incubated at 37°C in a humidified atmosphere containing 5% CO<sub>2</sub> for 12 h. The culture medium was removed, and the cells were washed with PBS. The cells were then sensitized with Anti-DNP IgE (cat. no. D8406; MilliporeSigma) diluted in DMEM containing 10% FBS to a final concentration of 100 ng/ml and incubated for 12 h at 37°C with 5% CO<sub>2</sub>. After sensitization, the IgE-containing medium was removed, and the cells were washed with PBS. The cells were then treated with various concentrations of PTFAb and incubated for 1 h at 37°C. The medium was then removed, and the cells were washed with Siraganian buffer. Degranulation was induced by a treatment with DNP-BSA diluted in Siraganian buffer to a final concentration of 20 ng/ml for 10 min. After stimulation, the cells were lysed using RIPA buffer (Cell Signaling). The lysates were centrifuged at 17,000 x g for 15 min at 4°C to obtain the cell lysates. The protein concentrations were determined using a DC protein assay, and the levels of Syk, MAPKs, and PI3K/AKT phosphorylation were analyzed by immunoblotting.

**Filaggrin expression analysis.** HaCaT cells were seeded at a density of  $1 \times 10^6$  cells per 100-mm dish and incubated for 12 h at 37°C in a humidified atmosphere containing 5% CO<sub>2</sub>. After incubation, the culture medium was removed, and the cells were washed with PBS. Except for the negative control group, the cells were treated with different PTFAb concentrations dissolved in culture medium containing TNF- $\alpha$  (5 ng/ml). The control group was treated with the culture medium without TNF- $\alpha$ . The cells were then incubated for an additional 24 h at 37°C in a 5% CO<sub>2</sub> incubator. Filaggrin protein expression was analyzed by immunoblotting.

**Hyaluronan synthase-related protein analysis.** HaCaT cells were seeded at  $1 \times 10^6$  cells per 100-mm dish and cultured for 12 h at 37°C in a humidified incubator containing 5% CO<sub>2</sub>. After incubation, the culture medium was removed, and the cells were washed with PBS, followed by serum starvation in serum-free DMEM for 6 h. The cells were treated with rhKGF (R&D Systems, Minneapolis, MN, USA) at 20 ng/ml as a positive control, or with the indicated concentrations of PTFAb, and incubated for an additional 12 h at 37°C under 5% CO<sub>2</sub>. Expression of hyaluronan synthesis-related proteins was analyzed by immunoblotting.

**Immunoblotting.** Immunoblotting analysis was performed as described elsewhere (28). The cells were lysed using RIPA buffer (Cell Signaling) and centrifuged at 17,000 x g for 15 min at 4°C. The total protein concentration in the supernatant was determined using a DC protein assay kit. The proteins (30–60  $\mu$ g per lane) were fractionated by 8–12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred electrophoretically to polyvinylidene fluoride (PVDF) membranes at 4°C. The membranes were blocked with 3% skim milk or BSA at room temperature for 2 h. Subsequently, the membranes were washed with PBS containing 0.05% Tween-20 and incubated with the primary antibodies against VAMP7 (dilution, 1:2,000), VAMP8 (dilution, 1:2,000), Syntaxin 1a (dilution, 1:1,000), Syntaxin

4 (dilution, 1:2,000), SNAP 23 (dilution, 1:1000), SNAP 25 (dilution, 1:2,000), phospho p38 MAPK (dilution, 1:1,000), p38 MAPK (dilution, 1:1,000), phospho ERK1/2 (dilution, 1:2,000), ERK 1/2 (dilution, 1:1,000), phospho JNK (dilution, 1:1,000), JNK (dilution, 1:1,000), phospho Syk (dilution, 1:1,000), Syk (dilution, 1:500), phospho PI3K (dilution, 1:1,000), PI3K (dilution, 1:1,000), phospho AKT (dilution, 1:1,000), AKT (dilution, 1:1,000), filaggrin (dilution, 1:500), HAS-2 (dilution, 1:2,000), HAS-3 (dilution, 1:1,000), and ICAM-1 (dilution, 1:1,000) at 4°C overnight.  $\beta$ -actin (dilution, 1:5,000) was incubated at room temperature for 30 min. The membranes were then exposed to horseradish peroxidase conjugated rabbit immunoglobulin G (dilution, 1:2,000) or mouse immunoglobulin G (dilution, 1:2,000) at room temperature for 1 h.  $\beta$ -actin was used as a loading control to confirm equal protein loading and for normalization of protein expression levels. Specifically, the levels of phosphorylated proteins were quantified as the ratio to their corresponding total protein levels. Normalization to  $\beta$ -actin was applied only for the quantification of total protein expression. The protein bands were visualized using a chemiluminescence substrate (cat. no. 2332632; ATTO, Tokyo, Japan) and detected with a chemiluminescence imaging system (LuminoGraph, ATTO, Tokyo, Japan).

**Statistical analysis.** All statistical analyses were performed using GraphPad Prism version 5.0 (GraphPad Software, Inc., La Jolla, CA, USA). The data are expressed as the mean  $\pm$  standard error of the mean (SEM). One-way ANOVA followed by Tukey's post hoc test was used for multiple group comparisons. Results in the present study are based on at least three independent experiments.  $P < 0.05$  was considered to indicate a statistically significant difference.

## Results

**Chemical composition of PTFAb.** The chemical composition of PTFAb was analyzed by GC/MS, which identified 13 components (Table I and Fig. 1). Among the identified components, methyl undecanoate (45.63%) had the highest content, followed in order by heneicosane (13.62%), tricosane (13.62%), sesamin (10.33%), 1,4-dimethoxybenzene (5.98%), tetracosane (4.79%), methyl anisate (1.79%), eicosanal (1.36%), 1-octen-3-ol (0.73%), methyl benzoate (0.71%),  $\beta$ -ionone (0.68%), nonanal (0.41%), and 3-oxo- $\alpha$ -ionone (0.35%) (Table I).

**Alteration in SNARE proteins linked to mast cell degranulation by PTFAb.** The PTFAb test concentration for biological analysis was first determined by observing the changes in cell viability in RBL-2H3 mast cells treated with PTFAb. Treatment with PTFAb (1–200  $\mu$ g/ml) showed a statistically significant decrease in cell viability compared to the control group at 200  $\mu$ g/ml, but no significant decrease was observed at 1–150  $\mu$ g/ml (Fig. 2A). Although there was no significant decrease in cell viability at 150  $\mu$ g/ml, the observed cell morphology appeared less uniformly rounded and slightly more irregular (Fig. S1). Therefore, the PTFAb test concentration for subsequent biological analysis was  $\leq 100$   $\mu$ g/ml, which did not affect the viability of RBL-2H3 cells. SNARE proteins, such as v-SNAREs (VAMP7 and VAMP8) and t-SNAREs (SNAP 23 and syntaxin 4), form

Table I. Chemical composition of *Paulownia tomentosa* flower absolute.

| Component name             | RT, min | RI       |            | Area, % | CAS no.    | (Refs.) |
|----------------------------|---------|----------|------------|---------|------------|---------|
|                            |         | Observed | Literature |         |            |         |
| 1. 1-Octen-3-ol            | 22.01   | 983      | 983        | 0.73    | 3391-86-4  | (59)    |
| 2. Methyl benzoate         | 29.50   | 1,098    | 1,098      | 0.71    | 93-58-3    | (60)    |
| 3. Nonanal                 | 29.91   | 1,106    | 1,106      | 0.41    | 124-19-6   | (61)    |
| 4. 1,4-Dimethoxybenzene    | 32.77   | 1,164    | 1,165      | 5.98    | 150-78-7   | (62)    |
| 5. Methyl anisate          | 46.22   | 1,383    | 1,386      | 1.79    | 121-98-2   | (62)    |
| 6. Methyl undecanoate      | 48.29   | 1,427    | 1,427      | 45.63   | 1731-86-8  | (63)    |
| 7. $\beta$ -Ionone         | 50.66   | 1,486    | 1,486      | 0.68    | 14901-07-6 | (64)    |
| 8. 3-Oxo- $\alpha$ -ionone | 55.97   | 1,658    | 1,665      | 0.35    | 79734-43-3 | (65)    |
| 9. Eicosanal               | 62.31   | 2,029    | 2,211      | 1.36    | 2400-66-0  | (66)    |
| 10. Heneicosane            | 62.94   | 2,101    | -          | 13.62   | 629-94-7   |         |
| 11. Tricosane              | 64.31   | 2,300    | -          | 13.62   | 638-67-5   |         |
| 12. Tetracosane            | 65.60   | 2,500    | -          | 4.79    | 646-31-1   |         |
| 13. Sesamin                | 67.53   | 2,753    | 3,150      | 10.33   | 607-80-7   | (67)    |

RT, retention time; RI, retention index (on a DB-5MS column). min: minutes. The identified compounds accounted for 100.00% of the total peak area.

trans-SNARE complexes to promote membrane fusion. These proteins help regulate the mast cell degranulation process and the release of inflammatory mediators, such as histamine and cytokines (4). The influence of PTFAb on the expression of SNARE proteins in RBL-2H3 mast cells was investigated by immunoblotting. PTFAb (0.1-100  $\mu$ g/ml) concentration-dependently inhibited the expression of the v-SNARE proteins VAMP7 (Fig. 2B and C) and VAMP8 in RBL-2H3 cells (Fig. 2B and D). The expression of VAMP7 and VAMP8 in RBL-2H3 cells decreased significantly after the treatment with PTFAb at 50-100  $\mu$ g/ml. The reduced expression in VAMP7 and VAMP8 showed a maximum at 100  $\mu$ g/ml of PTFAb 22.02 $\pm$ 8.61% (Fig. 2C) and 20.61 $\pm$ 7.40% (Fig. 2D) of the untreated control, respectively. On the other hand, the expression of the t-SNARE proteins (syntaxin 1a, syntaxin 4, SNAP 23, and SNAP 25) did not show significant changes after the treatment with PTFAb at 0.1-100  $\mu$ g/ml (Fig. S2).

*Effects of PTFAb on  $\beta$ -hexosaminidase and histamine release in RBL-2H3 mast cells.* Degranulation of activated mast cells results in the release of  $\beta$ -hexosaminidase and histamine. These mediators are mast cell degranulation markers and have been implicated in the pathogenesis of AD (1,33). The effects of PTFAb (0.1-100  $\mu$ g/ml) on DNP-BSA (20 ng/ml)-induced  $\beta$ -hexosaminidase and histamine release in anti-DNP IgE (200 ng/ml)-sensitized RBL-2H3 cells were examined to determine if PTFAb inhibits mast cell degranulation. DNP-BSA stimulation increased the levels of  $\beta$ -hexosaminidase (329.09 $\pm$ 4.77%) and histamine (193.02 $\pm$ 6.64%) compared to the anti-DNP IgE control (Fig. 3A and B, respectively). By contrast, the PTFAb treatment at 1-100  $\mu$ g/ml significantly reduced these levels in a concentration-dependent manner. In particular, PTFAb exhibited maximum inhibitory activity at 100  $\mu$ g/ml, reducing  $\beta$ -hexosaminidase and histamine to

146.06 $\pm$ 4.34% (Fig. 3A) and 111.63 $\pm$ 8.31% (Fig. 3B) of the anti-DNP IgE control, respectively.

*Effects of PTFAb on the degranulation-related signaling molecules in RBL-2H3 mast cells.* During mast cell degranulation and the release of inflammatory mediators, Syk phosphorylation, which accompanies the activation of the IgE receptor Fc $\epsilon$ RI (Fc epsilon receptor I), plays a key role in the initial signaling (34). PI3K/AKT and MAPK signaling pathways are also involved in this process (35). Thus, the effects of PTFAb on these key signaling molecules in mast cells were examined by observing the phosphorylation of Syk, PI3K/AKT, and MAPKs (p38, ERK1/2, and JNK) induced by DNP-BSA (20 ng/ml) in anti-DNP IgE antibody (100 ng/ml)-sensitized RBL-2H3 cells using immunoblotting to determine how PTFAb affects these key signaling molecules in mast cells. DNP-BSA significantly increased Syk phosphorylation (197.77 $\pm$ 13.46%) compared to the anti-DNP IgE control (Fig. 4A and B). On the other hand, treatment with PTFAb at 75 and 100  $\mu$ g/ml attenuated this level, and the maximum inhibitory effect was observed at 100  $\mu$ g/ml, reducing Syk phosphorylation to 91.13 $\pm$ 5.05% of the anti-DNP IgE control (Fig. 4B). In addition, the DNP-BSA treatment enhanced the phosphorylation levels of PI3K (143.67 $\pm$ 17.84%) and AKT (157.02 $\pm$ 5.79%) compared to the anti-DNP IgE control (Fig. 4C-E). The PTFAb treatments at 75 and 100  $\mu$ g/ml and at 50-100  $\mu$ g/ml significantly attenuated the increased levels of PI3K and AKT phosphorylation, respectively. The maximum inhibitory effects on the phosphorylation of these proteins were observed at 100 and 75  $\mu$ g/ml, with the PI3K and AKT phosphorylation levels reduced to 60.80 $\pm$ 6.24% (Fig. 4D) and 59.42 $\pm$ 9.37% of the anti-DNP IgE control group, respectively (Fig. 4E). The total protein levels of Syk, PI3K, and AKT normalized to  $\beta$ -actin are shown in Fig. S3, indicating that the observed effects were not due to changes in total protein expression. On the other hand, PTFAb

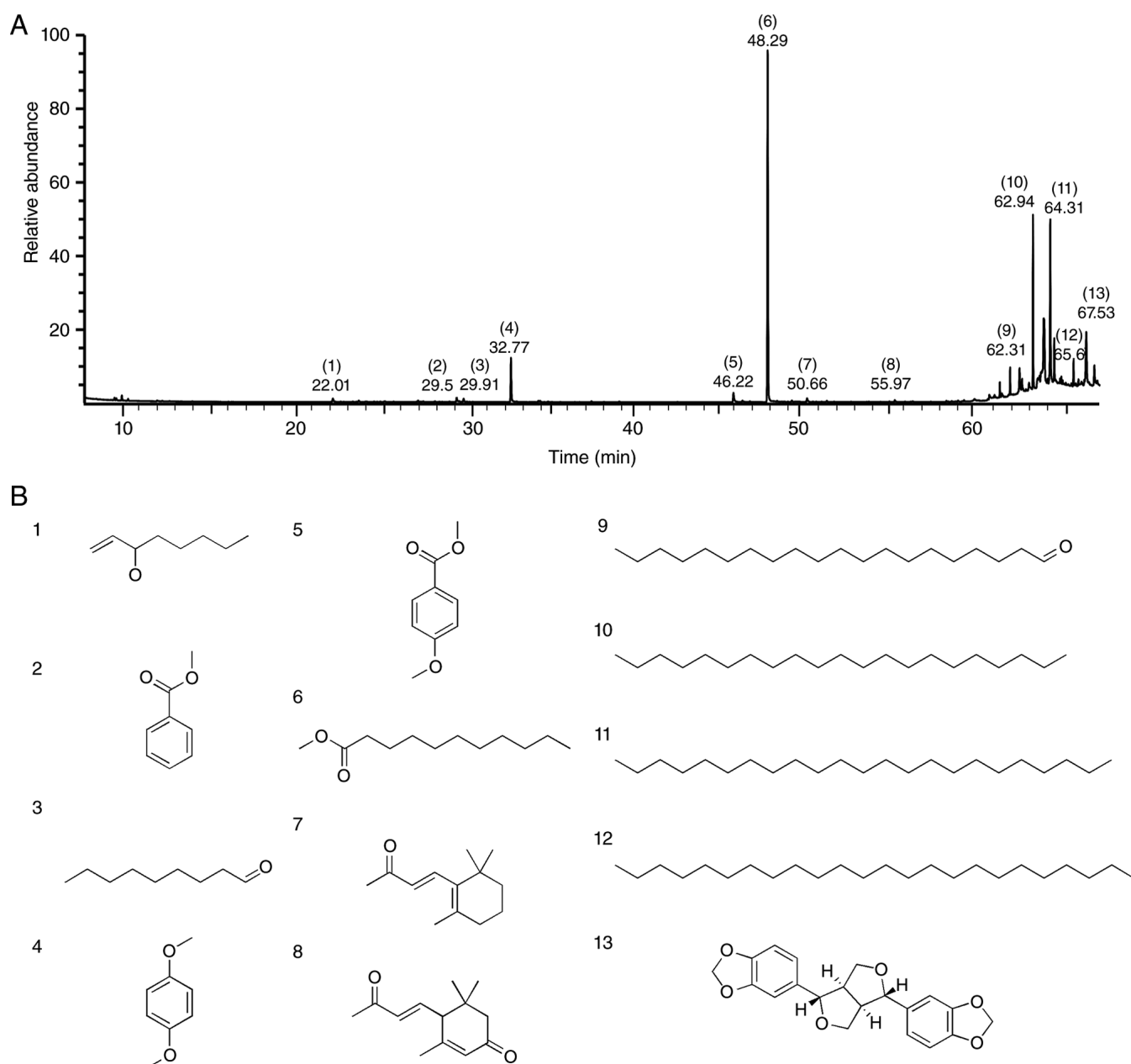


Figure 1. Gas chromatography/mass spectrometry analysis of *Paulownia tomentosa* flower absolute. (A) Total ion chromatogram showing the peaks corresponding to the 13 major compounds listed in Table I. The bracketed numbers and the numbers below them indicate the compound numbers and retention times, respectively. (B) Chemical structures of the 13 major compounds shown in panel A.

(0.1-100  $\mu\text{g/ml}$ ) did not affect the phosphorylation of MAPKs (p38, ERK1/2, and JNK) induced by DNP-BSA (20 ng/ml) in anti-DNP IgE antibody (100 ng/ml)-activated RBL-2H3 cells (Fig. S4).

To further investigate the involvement of Syk/PI3K and MAPK signaling pathways in the anti-degranulation activity of PTFab, pharmacological inhibitors targeting Syk, PI3K, p38, and ERK1/2 were evaluated. PTFab (50  $\mu\text{g/ml}$ ), piceatannol (a Syk inhibitor; 30  $\mu\text{M}$ ), and LY294002 (a PI3K inhibitor; 0.1  $\mu\text{M}$ ) significantly reduced anti-DNP IgE/DNP-induced  $\beta$ -hexosaminidase release (Fig. S5A). Co-treatment of PTFab with either piceatannol or LY294002 further reduced anti-DNP IgE/DNP-induced  $\beta$ -hexosaminidase release compared with PTFab treatment alone (Fig. S5B). In contrast, treatment with SB203580 increased anti-DNP IgE/DNP-induced

$\beta$ -hexosaminidase release (Fig. S5A), whereas co-treatment of PTFab with SB203580 partially attenuated the inhibitory effect of PTFab on anti-DNP IgE/DNP-induced  $\beta$ -hexosaminidase release (Fig. S5B). PD98059 alone or in combination with PTFab had little or no effect on anti-DNP IgE/DNP-induced  $\beta$ -hexosaminidase release (Fig. S5A and B).

**Effect of PTFab on the proliferation and migration of HaCaT cells.** The cytotoxic effects of PTFab on HaCaT cells were first assessed using a WST assay to determine its regenerative potential. The WST assay results showed that PTFab significantly enhanced cell viability at 250 and 500  $\mu\text{g/ml}$ , while concentrations at 1 to 100  $\mu\text{g/ml}$  showed only a slight, non-significant increase (Fig. 5A). Therefore, the entire concentration range (1-500  $\mu\text{g/ml}$ ) of PTFab was

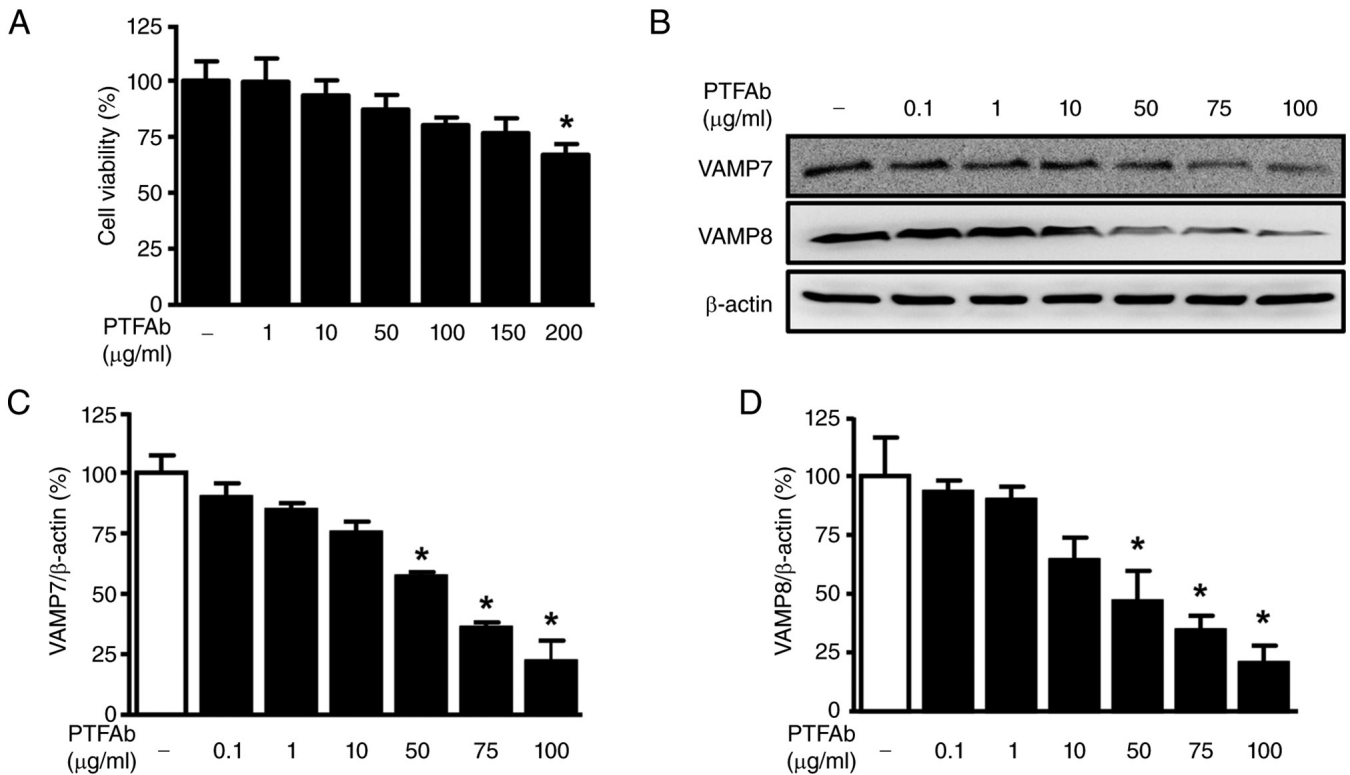


Figure 2. Effects of PTFAb on the expression of SNARE proteins in RBL-2H3 cells. (A) RBL-2H3 cell viability. RBL-2H3 cells were incubated in the presence or absence of PTFAb (1-200  $\mu\text{g/ml}$ ) for 24 h. The final DMSO concentration was maintained at 0.1% in all experimental groups, and control cells were treated with 0.1% DMSO as the vehicle control. The cell viability was analyzed using a WST assay ( $n=5$ ). \* $P<0.05$  compared with the vehicle control cells. (B) RBL-2H3 cells were incubated for 48 h in the presence or absence of PTFAb (0.1-100  $\mu\text{g/ml}$ ). The final DMSO concentration was maintained at 0.1% in all groups, including the vehicle control group. The cell lysates were immunoblotted with the indicated antibodies. (C and D) The graphs show the expression levels of the VAMP7 (C) and VAMP8 (D) proteins shown in panel B. The expression levels of VAMP7 and VAMP8 were quantified by normalization to  $\beta$ -actin (loading control) and presented as a percentage relative to the vehicle control group. \* $P<0.05$  vs. the vehicle control cells. DMSO, dimethyl sulfoxide; PTFAb, *Paulownia tomentosa* flower absolute; SNARE, soluble N-ethylmaleimide-sensitive factor attachment protein receptor; VAMP, vesicle-associated membrane protein; WST, water-soluble tetrazolium.

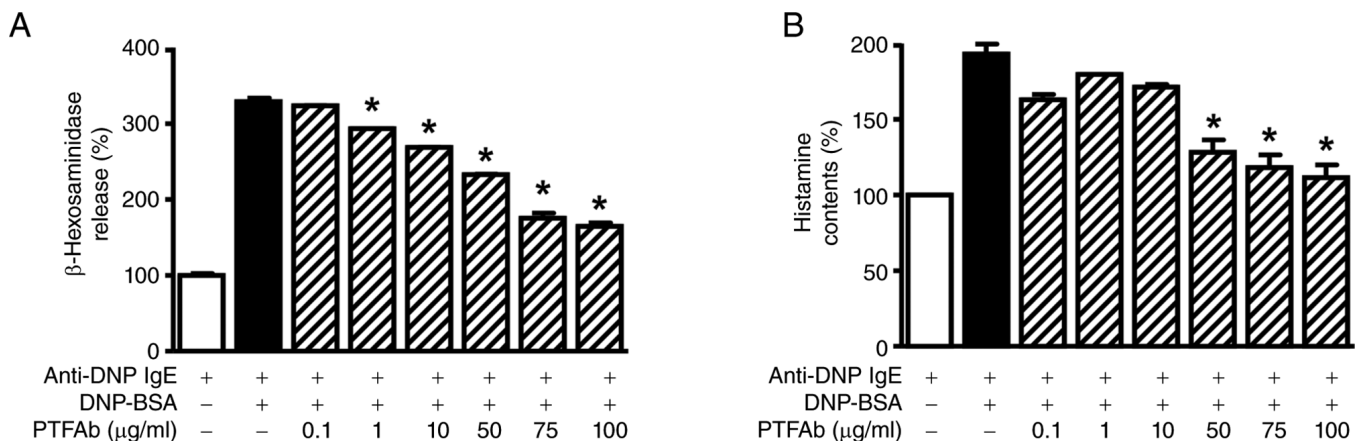


Figure 3. Effects of PTFAb on  $\beta$ -hexosaminidase and histamine release in IgE/DNP-stimulated RBL-2H3 cells. RBL-2H3 cells were incubated for 48 h in the presence or absence of PTFAb (0.1-100  $\mu\text{g/ml}$ ). The final DMSO concentration was maintained at 0.1% in all experimental groups. The cells were treated with anti-DNP IgE (200 ng/ml) for 10 h and then stimulated with DNP-BSA (20 ng/ml) for 1 h. The culture media were collected and centrifuged, and the levels of (A)  $\beta$ -hexosaminidase ( $n=3$ ) and (B) histamine ( $n=3$ ) in the supernatant (conditioned media) were measured using an enzyme immunoassay. The response in cells treated with anti-DNP IgE alone in the presence of 0.1% DMSO was considered 100%. \* $P<0.05$  vs. anti-DNP IgE/DNP-BSA-stimulated cells in the presence of 0.1% DMSO alone. DMSO, dimethyl sulfoxide; anti-DNP IgE, anti-dinitrophenyl immunoglobulin E; DNP-BSA, 2,4-dinitrophenyl-labeled bovine serum albumin; PTFAb, *Paulownia tomentosa* flower absolute.

used for further studies. BrdU assays showed that HaCaT cells proliferation was increased significantly by PTFAb at 250 and 500  $\mu\text{g/ml}$ , with the maximum increase occurred at 500  $\mu\text{g/ml}$

(143.98 $\pm$ 12.34% compared to the untreated control) (Fig. 5B). Furthermore, the cell migration assay results showed that a PTFAb treatment at 100-500  $\mu\text{g/ml}$  significantly promoted the

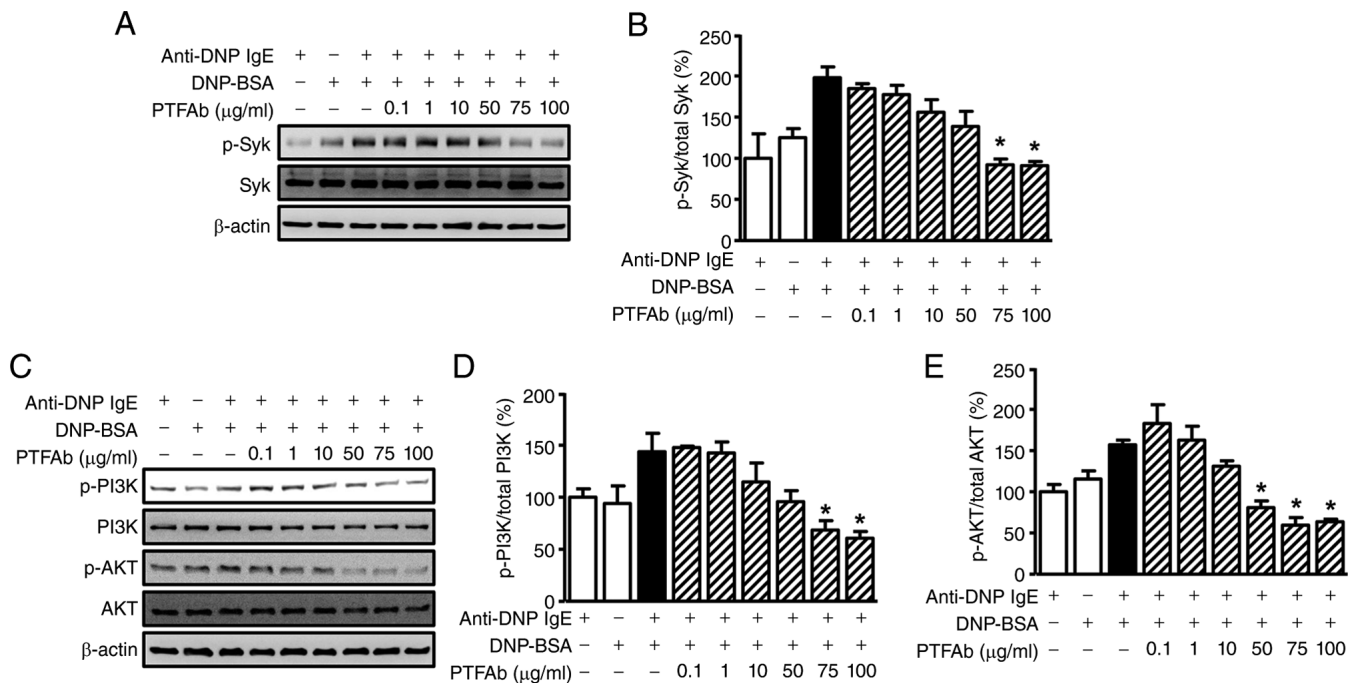


Figure 4. Effects of PTFab on Syk, PI3K, and AKT phosphorylation in RBL-2H3 cells. (A and B) Syk phosphorylation. (A) Representative image. RBL-2H3 cells were sensitized with anti-DNP IgE and subsequently stimulated with DNP-BSA to induce Syk phosphorylation. The cells were treated with PTFab (0.1-100 μg/ml), and the expression levels of phosphorylated Syk were analyzed by Western blotting (n=3). The final DMSO concentration was maintained at 0.1% in all experimental groups. (B) Quantification of the p-Syk levels normalized to the total Syk, presented as a percentage relative to anti-DNP IgE-sensitized cells treated with 0.1% DMSO alone. β-actin was used as a loading control to verify equal protein loading. \*P<0.05 vs. DNP-BSA-stimulated cells in the presence of anti-DNP IgE alone. (C-E) Phosphorylation of PI3K and AKT. (C) RBL-2H3 cells were sensitized with anti-DNP IgE and then stimulated with DNP-BSA to induce PI3K and AKT phosphorylation. The cells were treated with PTFab (0.1-100 μg/ml), and the expression levels of p-PI3K and p-AKT were analyzed by Western blotting (n=3 for each protein). The final DMSO concentration was maintained at 0.1% in all experimental groups. Quantification of the (D) p-PI3K and (E) p-AKT levels normalized to the respective total protein levels (total PI3K and total AKT), and presented as percentages relative to anti-DNP-IgE-sensitized cells treated with 0.1% DMSO alone. β-actin was used as a loading control to verify equal protein loading. \*P<0.05 vs. anti-DNP IgE/DNP-BSA-stimulated cells in the presence of 0.1% DMSO alone. DMSO, dimethyl sulfoxide; anti-DNP IgE, anti-dinitrophenyl immunoglobulin E; DNP-BSA, 2,4-dinitrophenyl-labeled bovine serum albumin; p-AKT, phosphorylated protein kinase B; p-PI3K, phosphorylated phosphoinositide 3-kinase; PTFab, *Paulownia tomentosa* flower absolute; p-Syk, phosphorylated spleen tyrosine kinase.

migration of HaCaT cells, peaking at  $840.00 \pm 52.46\%$  at the 500 μg/ml (Fig. 5C and D).

**Alteration of collagen synthesis in HaCaT cells by PTFab.** Collagen synthesis strengthens the integrity of the dermal and basement membranes, a key component of the skin barrier, supporting keratinocyte migration and proliferation (36). The effects of PTFab on collagen synthesis in HaCaT cells were evaluated using a sandwich enzyme-linked immunosorbent assay (ELISA). Treatment with PTFab (10-500 μg/ml) significantly increased type I collagen synthesis at 100 and 500 μg/ml, and type IV collagen synthesis at 500 μg/ml. Specifically, PTFab at 500 μg/ml had the most prominent effect, with synthesis levels reaching  $171.45 \pm 7.27$  and  $154.42 \pm 7.53\%$  for type I (Fig. 6A) and IV (Fig. 6B), respectively.

**Alteration of the skin barrier and hyaluronan production-related proteins in HaCaT cells by PTFab.** A reduction in the filaggrin levels and HASs (HAS-1, 2, and 3)-mediated HA synthesis impairs the structural stability and moisture retention of the skin barrier, contributing to skin barrier compromise of AD (37,38). Therefore, this study investigated the regulatory effects of PTFab on the expression of filaggrin and HAS in keratinocytes using immunoblotting. FLG protein expression in HaCaT cells was downregulated

by a TNF-α treatment to  $42.27 \pm 13.23\%$  of the untreated control. Nevertheless, treatment with PTFab at 250 and 500 μg/ml significantly restored FLG expression, which had been suppressed by TNF-α. In particular, 500 μg/ml PTFab had a maximum recovery effect ( $196.08 \pm 20.47\%$  of the untreated control) (Fig. 7A and B). In addition, this study observed the influence of PTFab on HAS expression in keratinocytes. The effects of PTFab on HAS-2 and HAS-3 expression were evaluated at concentrations ranging from 1 to 500 μg/ml (Fig. 7C-E). The PTFab treatment significantly increased the HAS-2 levels by  $377.11 \pm 55.21\%$  compared to the untreated control at 250 μg/ml (Fig. 7D). An increase ( $260.91 \pm 47.65\%$  of the untreated control) was also observed at 500 μg/ml, but did not have statistical significance. In contrast, HAS-3 expression was significantly upregulated at 250 and 500 μg/ml, with a maximum effect of  $443.78 \pm 61.56\%$  at 500 μg/ml (Fig. 7E).

**Effect of PTFab on ICAM-1 expression in HaCaT cells.** Increased expression of ICAM-1 in keratinocytes by inflammatory stimuli promotes the skin infiltration of immune cells and local inflammatory responses, which may be associated with abnormal skin barrier functions (15). Therefore, the changes in ICAM-1 protein expression were observed after a PTFab treatment of TNF-α-stimulated keratinocytes to

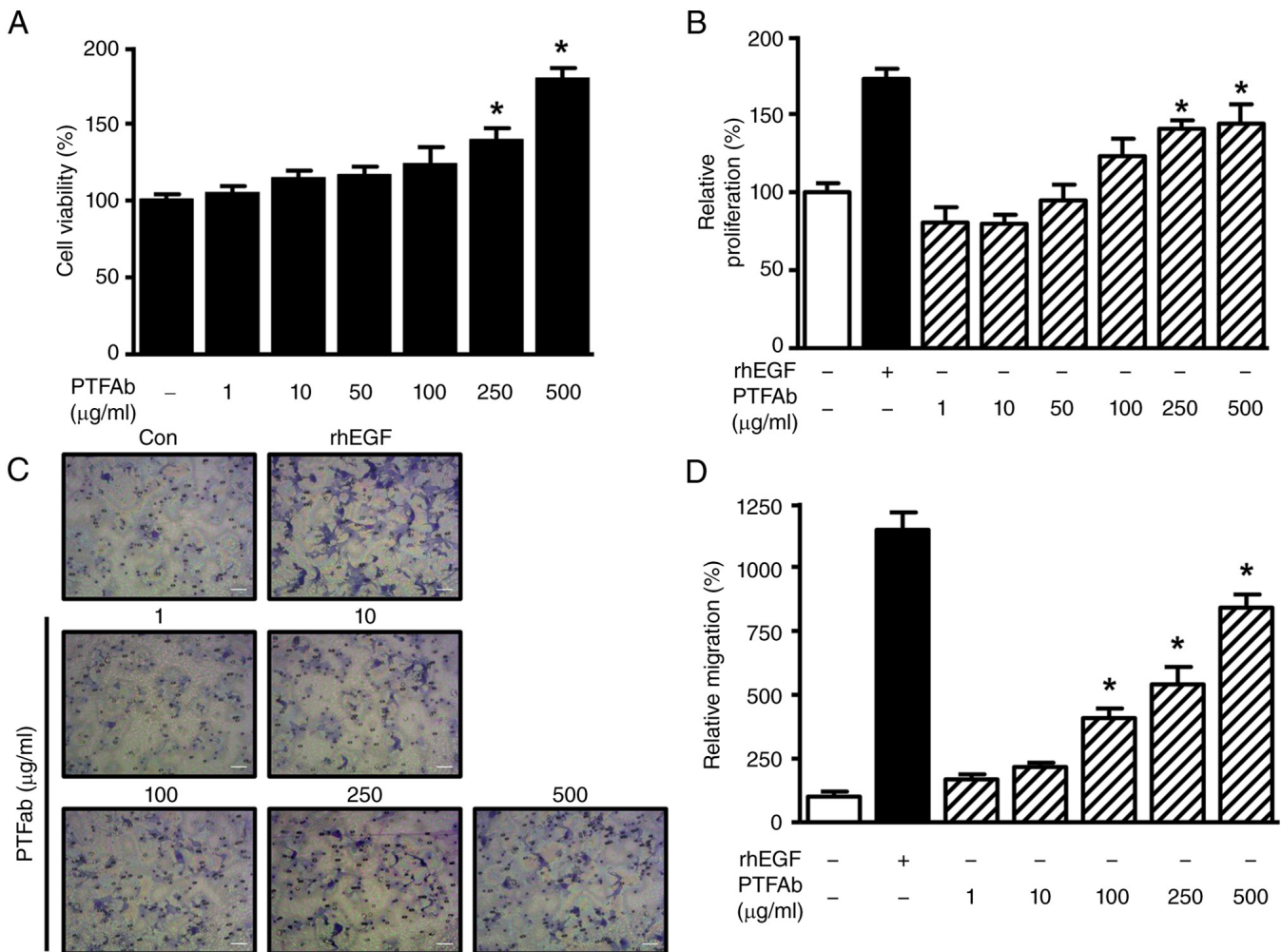


Figure 5. Effects of PTFab on the proliferation and migration of HaCaT cells. (A) Cell viability. HaCaT cells were incubated in the presence or absence of PTFab (1-500  $\mu\text{g/ml}$ ) for 24 h. The final DMSO concentration was maintained at 0.5% in all experimental groups, and control cells were treated with 0.5% DMSO as the vehicle control (-). The cell viability was analyzed using a WST assay (n=5). \* $P < 0.05$  compared with the untreated cells. (B) Proliferation. HaCaT cells were incubated in the presence or absence of PTFab (1-500  $\mu\text{g/ml}$ ) for 48 h. The final DMSO concentration was maintained at 0.5% in all groups, including the vehicle control group (-). Cell proliferation was analyzed using the BrdU incorporation assay (n=3). rhEGF (50 ng/ml)-induced proliferation was used as a positive control. The percentages represent the levels compared to that of the vehicle control cells, which are considered 100%. The results are presented as the means  $\pm$  SEMs. \* $P < 0.05$  compared to the quiescent state. (C and D) Migration. HaCaT cells were incubated in the presence or absence of PTFab (1-500  $\mu\text{g/ml}$ ) for 210 min. The final DMSO concentration was maintained at 0.5% in all experimental groups, including the vehicle control group. The cell migration levels were assessed using the Boyden chamber assay. (C) Representative images. The blue spots indicate migrating cells. (D) Statistical graph obtained from panel C. rhEGF (1 ng/ml)-induced migration was used as a positive control. The percentages represent the levels compared to the vehicle control, which are considered 100% (n=3). The results are presented as the means  $\pm$  SEMs. Scale bar=50  $\mu\text{m}$ . \* $P < 0.05$  compared with the vehicle control group. Con, vehicle control; BrdU, 5-bromo-2'-deoxyuridine; DMSO, dimethyl sulfoxide; PTFab, *Paulownia tomentosa* flower absolute; rhEGF, recombinant human epidermal growth factor; SEMs, standard errors of the means; WST, water-soluble tetrazolium.

determine if PTFab can alleviate skin inflammatory diseases through modulation of ICAM-1 expression. As shown in Fig. 8A and B, the TNF- $\alpha$  (10 ng/ml) treatment significantly increased ICAM-1 expression by  $179.08 \pm 9.70\%$  compared to the untreated control. Nevertheless, the PTFab treatment significantly reduced the expression levels at 250 and 500  $\mu\text{g/ml}$ . Specifically, the expression was maximally suppressed, reaching  $99.81 \pm 19.46\%$  of the untreated control (Fig. 8B).

## Discussion

AD is a chronic inflammatory skin disease that develops and progresses through the interaction of an imbalanced immune response and impaired epidermal barrier function.

These complex pathological characteristics are associated with persistent exacerbation and recurrent relapses (1). In particular, excessive secretion of inflammatory mediators due to mast cell activation and dysfunction of keratinocytes, which encompass the skin barrier, exacerbate the inflammatory responses and impede the restoration of skin homeostasis (1). Considering the characteristics of AD, simultaneously modulating the inflammatory responses and skin barrier function may be a practical therapeutic approach. The present study evaluated the anti-atopic potential of PTFab, an absolute-type essential oil, using mast cell and keratinocyte-based *in vitro* models. PTFab suppressed the mast cell responses associated with the secretion of inflammatory mediators while simultaneously promoting the keratinocyte responses involved in skin barrier formation

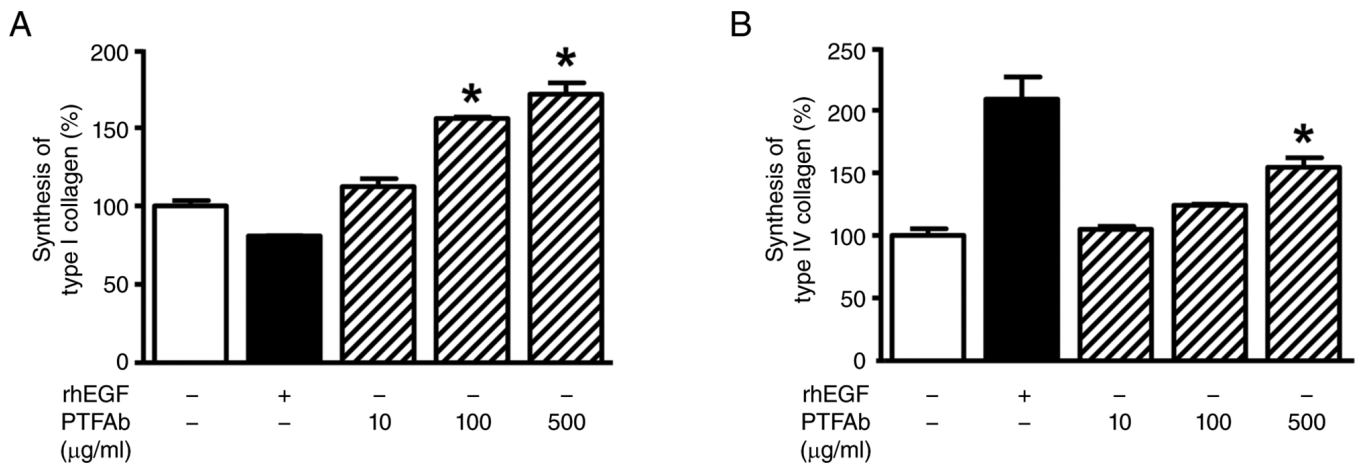


Figure 6. Effects of PTFab on the synthesis of type I and IV collagen in HaCaT cells. HaCaT cells were incubated in the presence or absence of PTFab (10, 100, and 500 µg/ml) for 48 h. The final DMSO concentration was maintained at 0.5% in all experimental groups, and control cells were treated with 0.5% DMSO as the vehicle control. The levels of (A) type I (n=3) and (B) IV collagen (n=3) in the conditioned media were quantified using a sandwich ELISA with the specific antibodies. The collagen synthesis levels are expressed as percentages of the levels in the conditioned media of vehicle control cells (-) considered for 100% (n=3). rhEGF (50 ng/ml)-induced response was used as a positive control. The results are presented as means ± SEMs. \*P<0.05 compared to the vehicle control cells. DMSO, dimethyl sulfoxide; ELISA, enzyme-linked immunosorbent assay; PTFab, *Paulownia tomentosa* flower absolute; rhEGF, recombinant human epidermal growth factor; SEMs, standard errors of the means.

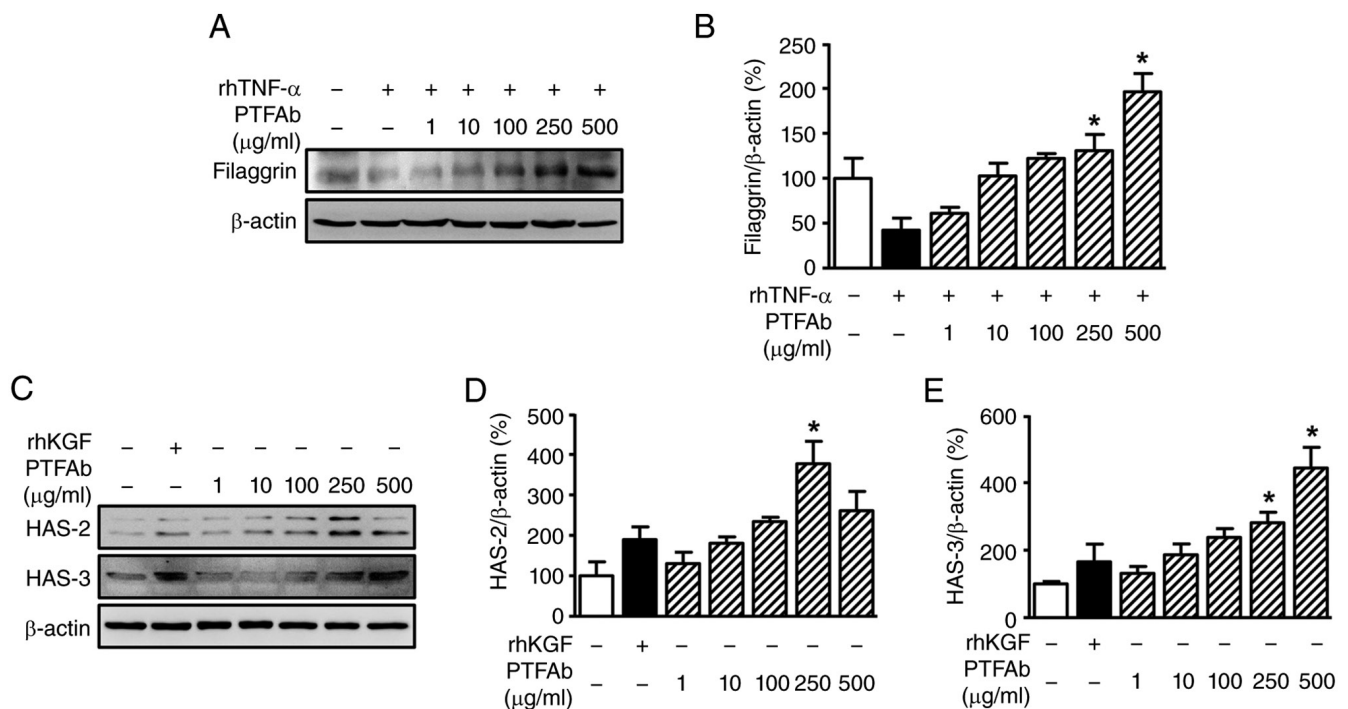


Figure 7. Effects of PTFab on expression of filaggrin and hyaluronan synthases in HaCaT cells. (A and B) Filaggrin expression. (A) Representative image. HaCaT cells were stimulated with TNF-α (5 ng/ml) to suppress filaggrin expression and then treated with PTFab (1-500 µg/ml). The final DMSO concentration was maintained at 0.5% in all experimental groups. The filaggrin protein levels were analyzed by immunoblotting (n=3). (B) Statistical graph. The relative expression of filaggrin was quantified by normalizing to β-actin (loading control) and presented as a percentage of the 0.5% DMSO alone-treated control group (vehicle control) (\*P<0.05 vs. TNF-α-stimulated cells in the presence of 0.5% DMSO alone). (C-E) Hyaluronan synthases. (C) Representative images. HaCaT cells were treated with PTFab (1-500 µg/ml), and rhKGF (20 ng/ml) was applied to induce HAS expression. The final DMSO concentration was maintained at 0.5% in all experimental groups. The protein levels of HAS-2 and HAS-3 were analyzed by Western blotting (n=3 for each protein). (D and E) Statistical graph. The relative expression levels of (D) HAS-2 and (E) HAS-3 were quantified by normalizing to β-actin (loading control) and presented as percentages relative to the 0.5% DMSO alone-treated vehicle control cells. rhKGF was used as a positive control. \*P<0.05 compared to 0.5% DMSO alone-treated control group. DMSO, dimethyl sulfoxide; HAS, hyaluronan synthase; PTFab, *Paulownia tomentosa* flower absolute; rhKGF, recombinant human keratinocyte growth factor; TNF-α, tumor necrosis factor-α.

and maintenance, suggesting that PTFab may be a promising functional material that can modulate multiple AD-related cellular responses in mast cells and keratinocytes.

Mast cell activation involves membrane fusion between pre-existing granules or vesicles and the plasma membrane, resulting in degranulation and the release of allergic

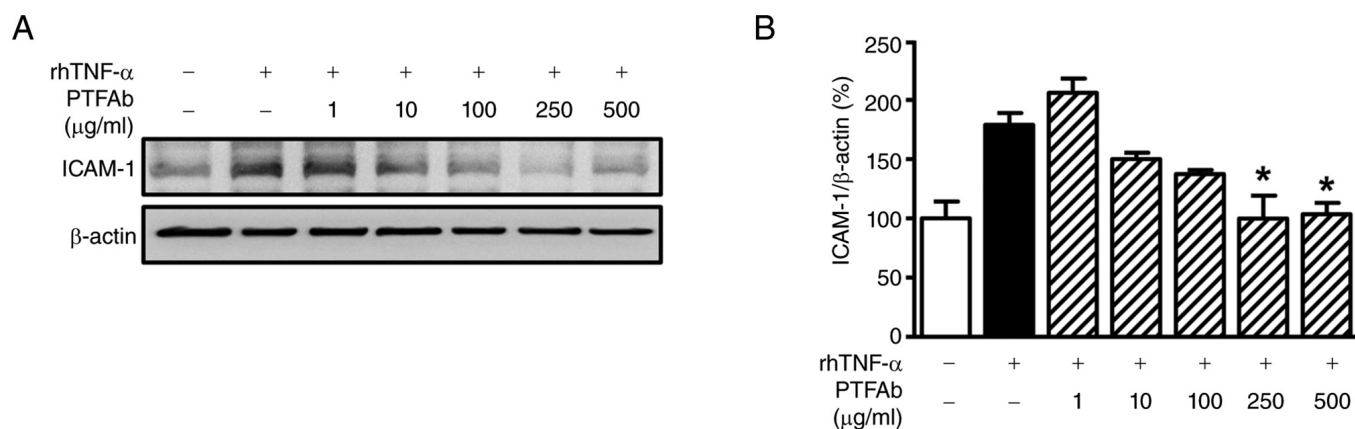


Figure 8. Effects of PTFAb on the expression of ICAM-1 protein in HaCaT cells. (A) HaCaT cells were stimulated with TNF- $\alpha$  (10 ng/ml) to induce ICAM-1 expression and then treated with PTFAb (1-500  $\mu$ g/ml). The final DMSO concentration was maintained at 0.5% in all experimental groups. The levels of ICAM-1 protein were analyzed by immunoblotting (n=3). (B) The relative expression of ICAM-1 was quantified by normalizing it to  $\beta$ -actin (loading control) and expressed as a percentage relative to the 0.5% DMSO alone-treated control group (vehicle control) (\* $P$ <0.05 vs. TNF- $\alpha$ -stimulated cells in the presence of 0.5% DMSO alone). DMSO, dimethyl sulfoxide; ICAM-1, intercellular adhesion molecule 1; PTFAb, *Paulownia tomentosa* flower absolute; TNF- $\alpha$ , tumor necrosis factor- $\alpha$ .

mediators stored within these granules (4). The membrane fusion process in mast cells is regulated by the SNARE complex, which is formed by the interaction between v-SNAREs and t-SNAREs (4). VAMP7 and VAMP8 are members of the v-SNARE proteins, and syntaxin 1a, syntaxin 4, SNAP 23, and SNAP 25 are members of the t-SNARE proteins. The interaction between these v-SNARE and t-SNARE proteins promotes mast cell degranulation. This mechanism was reported to be an important molecular target for the treatment of AD inflammatory lesions (29,39). Plant-derived substances inhibit mast cell degranulation by downregulating SNARE proteins and improve skin inflammatory symptoms in AD (39,40). Therefore, attenuating mast cell hyper-degranulation might be an important strategy for alleviating allergic inflammation. The present study found that PTFAb reduced VAMP7 and VAMP8 expression in RBL-2H3 cells, suggesting that PTFAb suppresses mast cell membrane fusion by interfering with SNARE complex formation. On the other hand, PTFAb did not suppress the expressions of syntaxin 1a, syntaxin 4, SNAP 23, and SNAP 25 in RBL-2H3 cells. Therefore, PTFAb may inhibit membrane fusion by selectively regulating the v-SNARE proteins VAMP7 and VAMP8, thereby reducing mast cell degranulation.

Excessive mast cell degranulation is a key contributor to the allergic inflammation observed in AD (41). When activated by allergens and IgE, mast cells release mediators such as histamine,  $\beta$ -hexosaminidase, and cytokines via degranulation, triggering inflammatory reactions (33,41). Among these,  $\beta$ -hexosaminidase and histamine are widely recognized as reliable indicators of mast cell degranulation because their release levels reflect the extent of mediator secretion (33). A literature survey showed that among 13 components identified from PTFAb, sesamin was reported to reduce histamine release and the production and secretion of pro-inflammatory cytokines in mast cells stimulated with IgE (42), suggesting that this constituent may contribute to the inhibitory effect of PTFAb on mast cell-derived inflammatory allergic reactions. In the present study, treatment with PTFAb markedly

reduced  $\beta$ -hexosaminidase and histamine release in RBL-2H3 cells sensitized with anti-DNP IgE upon DNP-BSA stimulation. Hence, PTFAb might suppress  $\beta$ -hexosaminidase and histamine release, which may contribute to the regulation of AD-related allergic responses.

Binding Fc $\epsilon$ RI and IgE activates mast cells and initiates the early activation of Syk, which then propagates signals to various downstream signaling pathways associated with the Fc $\epsilon$ RI-mediated degranulation and the production of allergic and inflammatory cytokines (6,34). Among these pathways, the PI3K/AKT axis plays a key role in inducing degranulation (33). In addition, the MAPK signaling pathway, represented by ERK1/2, JNK, and p38, was reported to regulate late-phase responses in the mast cells, including the regulation of inflammatory cytokine and chemokine expression, and degranulation (6,34). Therefore, targeting these signaling pathways may be a promising strategy for suppressing mast cell-mediated allergic inflammatory responses. A previous study reported that the inhibition of Syk-PI3K/AKT and MAPK (p38, JNK, and ERK1/2) phosphorylation by the plant extract was associated with the suppression of DNCB-IgE-mediated mast cell degranulation and inflammatory cytokine expression, probably ameliorating DNCB-induced AD-like lesions in animals (30). RBL-2H3 cells and DNCB-induced skin lesions are widely used as *in vitro* and *in vivo* models, respectively, for studying the mast cell-mediated allergic inflammatory responses and AD lesions (39). In the present study, the PTFAb treatment significantly reduced the levels of Syk, PI3K, and AKT phosphorylation in RBL-2H3 cells sensitized with anti-DNP IgE upon DNP-BSA stimulation, whereas no significant changes were observed in the phosphorylation of the MAPK family members, ERK1/2, JNK, and p38. Therefore, these findings suggest that PTFAb may be associated with reduced activation of the Syk-PI3K/AKT signaling pathway, rather than broad suppression of all downstream signaling pathways examined in this study.

In addition, to further investigate the potential contribution of Syk-PI3K/AKT and MAPK signaling to the

anti-degranulation activity of PTFAb, we evaluated the effects of pharmacological inhibitors targeting Syk, PI3K, and MAPKs on anti-DNP IgE/DNP-BSA-induced  $\beta$ -hexosaminidase release. Co-treatment of PTFAb with the Syk inhibitor piceatannol or the PI3K inhibitor LY294002 further reduced anti-DNP IgE/DNP-BSA-induced  $\beta$ -hexosaminidase release compared with PTFAb treatment alone. Although these additive effects do not establish that the activity of PTFAb is dependent on the Syk-PI3K/AKT pathway, they provide supportive, but not definitive, pharmacological evidence that this signaling axis may contribute to the observed effects of PTFAb. Interestingly, inhibition of p38 with SB203580 increased anti-DNP IgE/DNP-BSA-induced  $\beta$ -hexosaminidase release and partially attenuated the inhibitory effect of PTFAb, whereas inhibition of ERK1/2 with PD98059 had little effect either alone or in combination with PTFAb. Consistent with these observations, PTFAb did not significantly alter the phosphorylation of p38, ERK1/2, or JNK, suggesting that MAPK signaling may not represent a major molecular signaling pathway modulated by PTFAb. Collectively, these findings indicate that PTFAb is associated with reduced phosphorylation of Syk, PI3K, and AKT, whereas the inhibitor experiments provide only supportive, non-definitive evidence for the possible involvement of the Syk-PI3K/AKT signaling axis in its anti-degranulation activity of PTFAb. Further studies are needed to determine whether, and to what extent, this signaling pathway contributes to the anti-degranulation effects of PTFAb and to clarify the potential crosstalk between the Syk-PI3K/AKT and MAPK signaling pathways in the regulation of mast cell degranulation.

In addition to immune dysfunction leading to dysregulated allergic responses, AD is characterized by impaired skin barrier function (1). This impaired barrier function can be linked to abnormalities in the behavior of keratinocytes, the main cells in the epidermal layer of the skin, because these cells are essential for restoring the functional epidermal barrier through proliferation and migration (43). These processes may contribute to impaired epidermal renewal and delayed barrier restoration in AD. Interestingly, nonanal, a compound isolated from PTFAb, induces the expression of keratinocyte growth factor (KGF) and stimulates the proliferation and migration in dermal papilla cells (44). KGF promotes keratinocyte proliferation and migration (45). Based on these reports, it is suggested that PTFAb may exert stimulatory effects on HaCaT cell migration and proliferation. In the present study, PTFAb stimulated the proliferation and migration of HaCaT cells at non-cytotoxic concentrations (250–500  $\mu$ g/ml for proliferation and 100–500  $\mu$ g/ml for migration). These effects of PTFAb were mainly observed at relatively high concentrations. PTFAb is a crude botanical extract containing multiple constituents, and relatively high concentrations are often required to elicit measurable biological responses in *in vitro* studies. In addition, these concentrations of PTFAb showed no cytotoxicity, suggesting that the observed effects were not attributable to nonspecific cytotoxic responses. Furthermore, topical administration may allow relatively high local concentrations to be achieved at the site of application, but this possibility requires confirmation in appropriate *in vivo* studies. Nevertheless, the physiological/pharmacological relevance of these

concentrations remains to be established through appropriate *in vivo* studies.

Taken together, these findings suggest that PTFAb has the potential to modulate AD-related keratinocyte responses by promoting re-epithelialization and enhancing skin barrier-related functions.

Despite these promising biological activities, the active constituents responsible for the observed effects remain unclear. While GC/MS analysis identified 13 constituents in PTFAb, the present study did not isolate or identify the specific compounds responsible for the biological activities observed in the present study. Although several of the identified constituents, including sesamin and nonanal, have previously been reported to possess biological activities relevant to the present findings, their individual contributions were not experimentally verified. Therefore, the relationship between the chemical composition of PTFAb and its biological activities remains to be established. Because PTFAb is a complex mixture, its biological activities may result from the effects of individual constituents or from additive or synergistic interactions among multiple components. Further studies are required to examine fractionated extracts, isolate individual constituents, and elucidate the mechanisms underlying their activities to determine which constituents contribute to the cellular responses associated with the anti-AD potential of PTFAb.

The structural stability of skin tissue is provided by collagen, the predominant protein in the ECM, and influences key cellular functions such as adhesion, migration, and proliferation (46). Type I collagen is abundant in the interstitial layer and supports the structural integrity and elasticity of the skin, whereas type IV collagen in the basement membrane contributes to keratinocyte migration and proliferation, maintains the basement membrane structure, and mediates cell adhesion and signaling (47–50). In AD, reduced collagen levels have been linked to delayed wound healing, decreased elasticity, and increased skin dryness (50). Plant-derived extracts have the potential to support skin repair by stimulating collagen synthesis in keratinocytes (19). The results showed that PTFAb upregulated the production of collagen types I and IV in HaCaT cells. Hence, PTFAb may enhance ECM integrity and help stabilize skin barrier-related functions by promoting collagen production in keratinocytes.

FLG, IVL, and LOR, which are produced by keratinocytes, maintain the integrity of the skin barrier (7). Changes in the expression of these epidermal barrier-related proteins are widely known as a major cause of skin barrier dysfunction in AD (7). In particular, filaggrin mutations or deficiencies are reported to be a key contributor to the pathogenesis of AD, leading to a barrier dysfunction (51). In this context, studies have shown that FLG deficiency impairs barrier integrity, whereas restoring or upregulating FLG promotes barrier recovery from atopic, inflammatory, or chemically induced skin damage (51–53). These reports highlight the importance of regulating the barrier proteins in maintaining and restoring the epidermal barrier function in AD. In the present study, PTFAb enhanced FLG expression in HaCaT cells exposed to TNF- $\alpha$ , highlighting its potential to promote epidermal barrier recovery in AD. Future studies into its effects on IVL and LOR expression could further clarify the therapeutic potential of PTFAb in restoring the barrier function.

Hyaluronic acid (HA), a glycosaminoglycan component of the ECM, helps sustain skin moisture and elasticity through its strong affinity for water; hence, it is a key molecule in skin hydration maintenance (10,11). Its synthesis in keratinocytes is mediated by HAS enzymes (HAS-1, -2, and -3) (10). Maintaining proper skin moisture is essential for preserving the barrier integrity and the balance of skin functions, associated with a normal skin barrier function (54). HA ameliorated 2,4-dinitrofluorobenzene-induced AD-like lesions in mice (55). An emollient containing hydrolyzed collagen and HA improved the skin barrier function and enhanced hydration in AD, as shown in the clinical, *in vitro*, and *ex vivo* studies (56). These reports highlight its potential use in AD therapy. In the present study, PTFab increased HAS-2 and HAS-3 expression in HaCaT cells. Hence, PTFab may help enhance HAS (HAS-2 and HAS-3) expression in keratinocytes, probably improving skin hydration by increasing HA production. Overall, PTFab may promote cellular responses associated with wound healing, skin barrier function, and hydration in keratinocytes.

The interaction between keratinocytes, primary skin cells, and various immune cells is associated with recurrent and persistent inflammation in AD (1). Keratinocytes show increased expression of adhesion molecules like ICAM-1 in response to inflammatory stimuli. This process facilitates the infiltration and retention of T cells in the skin, contributing to skin barrier dysfunction and sustaining local inflammatory response (57). A previous study reported that TNF- $\alpha$  induces ICAM-1 in keratinocytes and ICAM-1 is upregulated in keratinocytes in inflammatory lesions of AD skin (58). In the present study, TNF- $\alpha$ , which induces an inflammatory condition, increased ICAM-1 expression, which was inhibited after PTFab stimulation. These findings suggest that PTFab may attenuate AD-linked inflammation, probably by reducing immune cell adhesion and infiltration at the keratinocyte level. In addition, PTFab inhibited histamine and  $\beta$ -hexosaminidase release from mast cells, highlighting its potential to suppress excessive immune activation during the early phase of allergic responses. Collectively, these findings suggest that PTFab may exert anti-inflammatory effects by regulating adhesion molecule expression and the release of inflammatory mediators. Therefore, PTFab may simultaneously regulate multiple mast cell- and keratinocyte-mediated pathways associated with AD-related cellular responses.

In conclusion, 13 compounds were identified from PTFab, showing that PTFab modulates the key cellular responses associated with the pathogenesis of AD. PTFab downregulated the expression of the v-SNARE proteins VAMP7 and VAMP8 in RBL-2H3 mast cells. PTFab also inhibited  $\beta$ -hexosaminidase and histamine release, and suppressed Syk-PI3K/AKT phosphorylation in RBL-2H3 cells under stimulation with anti-DNP IgE and DNP-BSA. In addition, in keratinocytes, PTFab promoted barrier-restorative functions by enhancing cell proliferation and migration, increasing type I and type IV collagen synthesis, restoring filaggrin expression reduced by TNF- $\alpha$ , upregulating HAS-2 and HAS-3 expression, and suppressing TNF- $\alpha$ -induced ICAM-1 expression. These findings demonstrate that PTFab enhances keratinocyte-mediated barrier repair and hydration while reducing inflammatory adhesion molecule expression. Collectively, these results

suggest that PTFab may modulate cellular processes involved in AD pathogenesis through multiple mechanisms, including the inhibition of mast cell degranulation and the enhancement of keratinocyte barrier recovery. Importantly, this study provides mechanistic evidence that PTFab simultaneously regulates two interdependent processes associated with AD: mast cell-mediated allergic inflammation and keratinocyte-driven epidermal barrier dysfunction, supporting its potential as a multi-target natural product candidate. These findings provide a scientific basis for further investigation of PTFab as a natural product-based material for AD-related research. Nevertheless, additional validation in animal models and clinical studies will be needed to clarify its therapeutic potential.

Several limitations of the present study should be considered when interpreting the findings. Although HaCaT cells were obtained from a documented commercial source and routinely tested for mycoplasma contamination, short tandem repeat (STR) profiling was not performed in our laboratory to independently confirm cell identity. Therefore, this limitation should be taken into account when interpreting the present results.

In addition, standard positive controls commonly used in mast cell- and AD-related models were not included in the present study. The primary objective of this study was to investigate the biological effects and underlying mechanisms of PTFab by comparing its effects with corresponding stimulated control groups, rather than to directly compare its efficacy with established anti-allergic or anti-AD agents. Therefore, the effects of PTFab were evaluated based on its ability to attenuate stimulus-induced cellular responses. Although positive controls (rhEGF or rhKGF) were included in selected HaCaT cell-based assays to validate specific keratinocyte responses, these assay-specific controls do not address the absence of standard positive controls for the mast cell- and AD-related assays and were not intended to serve as reference compounds for mast cell degranulation or AD-related immune responses. Future studies including appropriate positive controls for mast cell and AD models will further strengthen the comparative evaluation and validation of the efficacy of PTFab.

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## Availability of data and materials

The data generated in the present study may be requested from the corresponding author, and the raw GC/MS data generated during the current study are available from the corresponding author upon reasonable request.

## Authors' contributions

HML conceptualized the study. HML, KJW and DYK performed the formal analysis. DYY, YYK, DYK, JHB and JSY conducted the investigation. HML, KJW and DYY organized, managed, verified and prepared the experimental data for analysis. HML, KJW and DYY prepared the original draft of the manuscript. DYY, DYK, YYK, JHB and JSY reviewed the draft. HML and KJW reviewed and edited the manuscript. HML supervised the study and acquired funding. HML, KJW and DYY confirm the authenticity of all the raw data. All authors reviewed, discussed, edited, read and approved the final manuscript.

## Ethics approval and consent to participate

Not applicable.

## Patient consent for publication

Not applicable.

## Competing interests

The authors declare that they have no competing interests.

## References

1. Sroka-Tomaszewska J and Trzeciak M: Molecular mechanisms of atopic dermatitis pathogenesis. *Int J Mol Sci* 22: 4130, 2021.
2. Mohd Kasim VNK, Noble SM, Liew KY, Tan JW, Israf DA and Tham CL: Management of atopic dermatitis via oral and topical administration of herbs in murine model: A systematic review. *Front Pharmacol* 13: 785782, 2022.
3. Matsuda H, Nakamura S and Yoshikawa M: Degranulation inhibitors from medicinal plants in antigen-stimulated rat basophilic leukemia (RBL-2H3) cells. *Chem Pharm Bull (Tokyo)* 64: 96-103, 2016.
4. Woska JR Jr and Gillespie ME: SNARE complex-mediated degranulation in mast cells. *J Cell Mol Med* 16: 649-656, 2012.
5. Hada M, Nishi K, Ishida M, Onda H, Nishimoto S and Sugahara T: Inhibitory effect of aqueous extract of *Cuminum cyminum* L. seed on degranulation of RBL-2H3 cells and passive cutaneous anaphylaxis reaction in mice. *Cytotechnology* 71: 599-609, 2019.
6. McCarty MF, Lerner A, DiNicolantonio JJ and Benavoli C: Nutraceuical aid for allergies-strategies for down-regulating mast cell degranulation. *J Asthma Allergy* 14: 1257-1266, 2021.
7. Furue M: Regulation of filaggrin, loricrin, and involucrin by IL-4, IL-13, IL-17A, IL-22, AHR, and NRF2: Pathogenic implications in atopic dermatitis. *Int J Mol Sci* 21: 5382, 2020.
8. Proksch E, Brandner JM and Jensen JM: The skin: An indispensable barrier. *Exp Dermatol* 17: 1063-1072, 2008.
9. Potekhaev NN, Borzykh OB, Medvedev GV, Pushkin DV, Petrova MM, Petrov AV, Dmitrenko DV, Karpova EI, Demina OM and Shnayder NA: The role of extracellular matrix in skin wound healing. *J Clin Med* 10: 5947, 2021.
10. Papakonstantinou E, Roth M and Karakiulakis G: Hyaluronic acid: A key molecule in skin aging. *Dermatoendocrinol* 4: 253-258, 2012.
11. Evrard C, Lambert de Rouvroit C and Poumay Y: Epidermal hyaluronan in barrier alteration-related disease. *Cells* 10: 3096, 2021.
12. Diller RB and Tabor AJ: The role of the extracellular matrix (ECM) in wound healing: A review. *Biomimetics (Basel)* 7: 87, 2022.
13. Choudhary V, Choudhary M and Bollag WB: Exploring skin wound healing models and the impact of natural lipids on the healing process. *Int J Mol Sci* 25: 3790, 2024.
14. Rerknimitr P, Otsuka A, Nakashima C and Kabashima K: The etiopathogenesis of atopic dermatitis: Barrier disruption, immunological derangement, and pruritus. *Inflamm Regen* 37: 14, 2017.
15. Singer KH, Tuck DT, Sampson HA and Hall RP: Epidermal keratinocytes express the adhesion molecule intercellular adhesion molecule-1 in inflammatory dermatoses. *J Invest Dermatol* 92: 746-750, 1989.
16. Matsunaga T, Katayama I, Yokozeki H and Nishioka K: ICAM-1 expression on keratinocytes in mechanically-injured skin of a patient with atopic dermatitis. *J Dermatol Sci* 12: 219-226, 1996.
17. Man G, Hu LZ, Elias PM and Man MQ: Therapeutic benefits of natural ingredients for atopic dermatitis. *Chin J Integr Med* 24: 308-314, 2018.
18. Valdivieso-Ugarte M, Gomez-Llorente C, Plaza-Díaz J and Gil Á: Antimicrobial, antioxidant, and immunomodulatory properties of essential oils: A systematic review. *Nutrients* 11: 2786, 2019.
19. Kim DY, Won KJ, Kim YY, Yoo DY and Lee HM: Potential wound healing and anti-melanogenic activities in skin cells of *Aralia elata* (Miq.) Seem. flower essential oil and its chemical composition. *Pharmaceutics* 16: 1008, 2024.
20. Chung IM, Kim EH, Jeon HS and Moon HI: Protective effects of isoatroplicolide tiglactate from *Paulownia coreana* against glutamate-induced neurotoxicity in primary cultured rat cortical cells. *Nat Prod Commun* 5: 851-852, 2010.
21. Si CL, Kim JK, Kwon DJ and Bae YS: Phenylpropanoid glycosides of *Paulownia coreana* Uyekii leaves. *J Korean Wood Sci Technol* 34: 78-82, 2006.
22. Kim TJ: Korean resources plants (IV). Seoul National University Press, Seoul, pp76-77, 1996.
23. Kim NK, Kim MH, Yoon CS and Choi SW: Studies on the anti-inflammatory activity of *Paulownia coreana* Uyekii leaf extract. *J Soc Cosmet Sci Korea* 32: 241-247, 2006.
24. Jin Q, Lee C, Lee JW, Lee D, Kim Y, Hong JT, Kim JS, Kim JH, Lee MK and Hwang BY: Geranylated flavanones from *Paulownia coreana* and their inhibitory effects on nitric oxide production. *Chem Pharm Bull (Tokyo)* 63: 384-387, 2015.
25. Teng Y, Zhong H, Yang X, Tao X and Fan Y: Current and emerging therapies for atopic dermatitis in the elderly. *Clin Interv Aging* 18: 1641-1652, 2023.
26. International Union for Conservation of Nature: The IUCN red list of threatened species. <https://www.iucnredlist.org>. Accessed March, 2026.
27. National Institute of Biological Resources (NIBR): Korean Red Data Book of Vascular Plants (2021). <https://species.nibr.go.kr>. Accessed March, 2026.
28. Kim MJ, Won KJ, Kim DY, Won YR, Kim NY, Lee DK, Hong BS and Lee HM: Skin wound healing and anti-wrinkle-promoting in vitro biological activities of *Caragana sinica* flower absolute and its chemical composition. *Pharmaceutics (Basel)* 16: 235, 2023.
29. Yang Y, Kong B, Jung Y, Park JB, Oh JM, Hwang J, Cho JY and Kweon DH: Soluble N-ethylmaleimide-sensitive factor attachment protein receptor-derived peptides for regulation of mast cell degranulation. *Front Immunol* 9: 725, 2018.
30. Lee HP, Choi W, Kwon KW, You L, Rahmawati L, Luong VD, Kim W, Lee BH, Lee S, Kim JH and Cho JY: Inhibitory effects of *Grewia tomentosa* Juss. on IgE-mediated allergic reaction and DNCB-induced atopic dermatitis. *Plants (Basel)* 11: 2540, 2022.
31. Bairoch A: The cellosaurus, a cell-line knowledge resource. *J Biomol Tech* 29: 25-38, 2018.
32. Bae WB, Kim EH, Kim MJ and Yang SA: Inhibitory effects of *Broussonetia kazinoki* twig extract on allergic inflammatory reactions in TNF- $\alpha$ /IFN- $\gamma$ -stimulated HaCaT and IgE-sensitized RBL-2H3 cells. *Food Sci Preserv* 31: 307-314, 2024.
33. Bao Y, Wang S, Gao Y, Zhang W, Jin H, Yang Y and Li J: MicroRNA-126 accelerates IgE-mediated mast cell degranulation associated with the PI3K/Akt signaling pathway by promoting  $Ca^{2+}$  influx. *Exp Ther Med* 16: 2763-2769, 2018.
34. Siraganian RP, de Castro RO, Barbu EA and Zhang J: Mast cell signaling: The role of protein tyrosine kinase Syk, its activation and screening methods for new pathway participants. *FEBS Lett* 584: 4933-4940, 2010.
35. Rivera J and Gilfillan AM: Molecular regulation of mast cell activation. *J Allergy Clin Immunol* 117: 1214-1226, 2006.
36. Mathew-Steiner SS, Roy S and Sen CK: Collagen in wound healing. *Bioengineering (Basel)* 8: 63, 2021.
37. Levin J, Friedlander SF and Del Rosso JQ: Atopic dermatitis and the stratum corneum: Part I: The role of filaggrin in the stratum corneum barrier and atopic skin. *J Clin Aesthet Dermatol* 6: 16-22, 2013.

38. Malaisse J, Bourguignon V, De Vuyst E, Lambert de Rouvroit C, Nikkels AF, Flamion B and Poumay Y: Hyaluronan metabolism in human keratinocytes and atopic dermatitis skin is driven by a balance of hyaluronan synthases 1 and 3. *J Invest Dermatol* 134: 2174-2182, 2014.
39. Kim DY, Won KJ, Hwang DI, Lee SY, Choi IH, Kim B and Lee HM: Essential oil from *Chrysanthemum boreale* flowers modulates SNARE protein-linked mast cell response and skin barrier proteins and ameliorates atopic dermatitis-like lesions in mice. *Hortic Environ Biotechnol* 63: 287-298, 2022.
40. Yun Y, Kim K, Choi I and Ko SG: Topical herbal application in the management of atopic dermatitis: A review of animal studies. *Mediators Inflamm* 2014: 752103, 2014.
41. Liu FT, Goodarzi H and Chen HY: IgE, mast cells, and eosinophils in atopic dermatitis. *Clin Rev Allergy Immunol* 41: 298-310, 2011.
42. Li LC, Piao HM, Zheng MY, Lin ZH, Li G and Yan GH: Sesamin attenuates mast cell-mediated allergic responses by suppressing the activation of p38 and nuclear factor- $\kappa$ B. *Mol Med Rep* 13: 536-542, 2016.
43. Wikramanayake TC, Stojadinovic O and Tomic-Canic M: Epidermal differentiation in barrier maintenance and wound healing. *Adv Wound Care (New Rochelle)* 3: 272-280, 2014.
44. Park S, Kang W, Choi D, Son B and Park T: Nonanal stimulates growth factors via cyclic adenosine monophosphate (cAMP) signaling in human hair follicle dermal papilla cells. *Int J Mol Sci* 21: 8054, 2020.
45. Putnins EE, Firth JD, Lohachitranont A, Uitto VJ and Larjava H: Keratinocyte growth factor (KGF) promotes keratinocyte cell attachment and migration on collagen and fibronectin. *Cell Adhes Commun* 7: 211-221, 1999.
46. Brett D: A review of collagen and collagen-based wound dressings. *Wounds* 20: 347-356, 2008.
47. O'Toole EA: Extracellular matrix and keratinocyte migration. *Clin Exp Dermatol* 26: 525-530, 2001.
48. Boudko SP, Danyelych N, Hudson BG and Pedchenko VK: Basement membrane collagen IV: Isolation of functional domains. *Methods Cell Biol* 143: 171-185, 2018.
49. Motter Catarino C, Kaiser K, Baltazar T, Motter Catarino L, Brewer JR and Karande P: Evaluation of native and non-native biomaterials for engineering human skin tissue. *Bioeng Transl Med* 7: e10297, 2022.
50. Szalus K and Trzeciak M: The role of collagens in atopic dermatitis. *Int J Mol Sci* 25: 7647, 2024.
51. Moosbrugger-Martinz V, Leprince C, Méchin MC, Simon M, Blunder S, Gruber R and Dubrac S: Revisiting the roles of filaggrin in atopic dermatitis. *Int J Mol Sci* 23: 5318, 2022.
52. Dang NN, Pang SG, Song HY, An LG and Ma XL: Filaggrin silencing by shRNA directly impairs the skin barrier function of normal human epidermal keratinocytes and then induces an immune response. *Braz J Med Biol Res* 48: 39-45, 2015.
53. Hsu CY, Lecland N, Pendaries V, Viodé C, Redoules D, Paul C, Merdes A, Simon M and Bierkamp C: Stabilization of microtubules restores barrier function after cytokine-induced defects in reconstructed human epidermis. *J Dermatol Sci* 91: 87-96, 2018.
54. Bonté F: Skin moisturization mechanisms: New data. *Ann Pharm Fr* 69: 135-141, 2011.
55. Zheng BW, Wang BY, Xiao WL, Sun YJ, Yang C and Zhao BT: Different molecular weight hyaluronic acid alleviates inflammation response in DNFB-induced mice atopic dermatitis and LPS-induced RAW 264.7 cells. *Life Sci* 301: 120591, 2022.
56. Lee YI, Lee SG, Kim J, Choi S, Jung I and Lee JH: Proteoglycan combined with hyaluronic acid and hydrolyzed collagen restores the skin barrier in mild atopic dermatitis and dry, eczema-prone skin: A pilot study. *Int J Mol Sci* 22: 10189, 2021.
57. Griffiths CE and Nickoloff BJ: Keratinocyte intercellular adhesion molecule-1 (ICAM-1) expression precedes dermal T lymphocytic infiltration in allergic contact dermatitis (Rhus dermatitis). *Am J Pathol* 135: 1045-1053, 1989.
58. Griffiths CE, Voorhees JJ and Nickoloff BJ: Characterization of intercellular adhesion molecule-1 and HLA-DR expression in normal and inflamed skin: Modulation by recombinant gamma interferon and tumor necrosis factor. *J Am Acad Dermatol* 20: 617-629, 1989.
59. Klesk K, Qian M and Martin RR: Aroma extract dilution analysis of cv. meeker (*Rubus idaeus* L.) red raspberries from Oregon and Washington. *J Agric Food Chem* 52: 5155-5161, 2004.
60. Wang Q, Yang Y, Zhao X, Zhu B, Nan P, Zhao J, Wang L, Chen F, Liu Z and Zhong Y: Chemical variation in the essential oil of *Ephedra sinica* from Northeastern China. *Food Chem* 98: 52-58, 2006.
61. Bozin B, Mimicá-Dukic N, Simin N and Anackov G: Characterization of the volatile composition of essential oils of some lamiaceae spices and the antimicrobial and antioxidant activities of the entire oils. *J Agric Food Chem* 54: 1822-1828, 2006.
62. Tsuge S, Ohtan H and Watanabe C: Pyrolysis-GC/MS data book of synthetic polymers. Elsevier, pp420, 2011.
63. Pino JA, Mesa J, Muñoz Y, Martí MP and Marbot R: Volatile components from mango (*Mangifera indica* L.) cultivars. *J Agric Food Chem* 53: 2213-2223, 2005.
64. Wang Y, Finn C and Qian MC: Impact of growing environment on chikasa blackberry (*Rubus* L.) aroma evaluated by gas chromatography olfactometry dilution analysis. *J Agric Food Chem* 53: 3563-3571, 2005.
65. Jerković I, Tuberso CIG, Gugić M and Bubalo D: Composition of sulla (*Hedysarum coronarium* L.) honey solvent extractives determined by GC/MS: Norisoprenoids and other volatile organic compounds. *Molecules* 15: 6375-6385, 2010.
66. Naka H, van Vang L, Inomata SI, Ando T, Kimura T, Honda H, Tsuchida K and Sakurai H: Sex pheromone of the persimmon fruit moth, *Stathmopoda masinissa*: Identification and laboratory bioassay of (4E, 6Z)-4,6-hexadecadien-1-ol derivatives. *J Chem Ecol* 29: 2447-2459, 2003.
67. National Institute of Standards and Technology (NIST): NIST Mass Spectrometry Data Center., NIST Gaithersburg, MD, 2014.



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