BIOMOLECULES & THERAPEUTICS

**Original Article**
*Biomol Ther 33(3), 510-517 (2025)*

# Hederacoside C Modulates EGF-Induced MUC5AC Mucin Gene Expression by Regulating the MAPK Signaling Pathway in Human Airway Epithelial Cells

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## Abstract

This study aimed to evaluate the potential of hederacoside C, an active compound isolated from *Hedera helix*, which has been used for managing inflammatory respiratory diseases, in attenuating epidermal growth factor (EGF)-induced airway MUC5AC mucin gene expression. Human pulmonary mucoepidermoid NCI-H292 cells were pretreated with hederacoside C for 30 min and subsequently stimulated with EGF for 24 h. The study also examined the effect of hederacoside C on the EGF-induced mitogen-activated protein kinase (MAPK) signaling pathway. The results showed that hederacoside C inhibited MUC5AC mucin mRNA expression and the production of mucous glycoproteins by suppressing the phosphorylation of the EGF receptor (EGFR), as well as the phosphorylation of MAPK/extracellular signal-regulated kinase (ERK) 1/2 (MEK1/2), p38 MAPK, ERK 1/2 (p44/42), and the nuclear expression of specificity protein-1 (Sp1). These findings suggest that hederacoside C has the potential to reduce EGF-induced mucin gene expression by inhibiting the EGFR-MAPK-Sp1 signaling pathway in NCI-H292 cells.

**Key Words:** Hederacoside C, MUC5AC, EGF, MAPK

## INTRODUCTION

Mucus, a thin gel-like layer on the luminal surface of the respiratory tract, contains water, ions, and various molecules, and exerts antioxidant and antimicrobial effects. Under normal physiological conditions, airway mucus plays a crucial role in protecting the pulmonary epithelium from damage caused by viruses, inhaled particles, irritating gases, and bacteria (Mann *et al*., 2022). However, excessive mucus production or secretion, arising from changes in mucin quantity or quality, can compromise the host defense system, contributing to increased morbidity and mortality in conditions such as chronic obstructive pulmonary disease (COPD), asthma, and cystic fibrosis (CF) (Ryu *et al*., 2023; Shah *et al*., 2023). The complex role of mucus in the pulmonary system highlights the importance of regulating its production and secretion to maintain respiratory health. Mucins, the major mucous glycoproteins in mucus, are responsible for mucus' viscoelasticity. Among the various human mucin subtypes, MUC2, MUC5AC, MUC5B, and MUC6 are the primary gel-forming, secreted mucins. MUC2 is predominantly found in the intestines but is also expressed at low levels in the lungs. MUC5AC and MUC6 are gastric mucins, with MUC5AC abundant in the lungs, while MUC6 is not. MUC5AC is the predominant secreted mucin in the airways, while MUC5B is a glandular mucin present in the tracheobronchial submucosal glands (Vestbo and Hogg, 2006; Evans and Koo, 2009; Allinson *et al*., 2016).

In the human lung, MUC5AC is primarily localized to epithelial cells on the airway surface, expressed in goblet cells at the luminal surface of healthy airways. It is the most abundant gel-forming mucin in asthma, COPD, and CF. While its levels are low in stable disease, they increase approximately ten-

**Open Access** **https://doi.org/10.4062/biomolther.2025.054**



**Received** Mar 24, 2025 **Revised** Apr 1, 2025 **Accepted** Apr 4, 2025
**Published Online** Apr 30, 2025

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fold during exacerbations (Evans and Koo, 2009; Kim *et al*., 2023a; Abrami *et al*., 2024).

Traditional treatments for controlling mucus production and secretion include agents like letocysteine, hypertonic saline, erdosteine, S-carboxymethyl cysteine, 2-mercaptoethane sulfonate sodium, ambroxol, bromhexine, glyceryl guaiacolate, N-acetyl L-cysteine (NAC), azithromycin, mannitol dry powder, dornase alfa, myrtol, and sobrerol. However, these treatments may have drawbacks, such as rebound over-secretion of mucus or irritation of the respiratory tract lining (Li *et al*., 2020). Therefore, there is a clear need for novel agents that can more effectively regulate mucin biosynthesis and/or degradation. Research suggests that natural products isolated from medicinal plants, which have historically been used for treating inflammation, may offer potential in regulating the abnormal secretion and/or production of MUC5AC mucin in pulmonary diseases.

Over the past two decades, numerous studies have investigated the effects of natural products on MUC5AC mucin gene expression (Kim *et al*., 2012; Ryu *et al*., 2013, 2014; Seo *et al*., 2014; Sikder *et al*., 2014; Lee *et al*., 2015; Kim *et al*., 2016; Li *et al*., 2020; Hossain *et al*., 2022a, 2022b; Kim *et al*., 2023a; Ryu *et al*., 2023). This research direction offers the potential for developing new therapeutic candidates to control airway mucin secretion and production, creating new opportunities for managing inflammatory pulmonary diseases. The exploration of natural products in drug discovery highlights the value of combining traditional knowledge with modern scientific methods (Hossain *et al*., 2022a, 2022b; Huh *et al*., 2023; Hwang *et al*., 2023; Jang *et al*., 2023; Kim *et al*., 2023b, 2023c; Ko *et al*., 2023; Lee *et al*., 2023b; Ryu *et al*., 2023).

According to the literature, hederacoside C (Fig. 1), a monodesmoside isolated from *Hedera helix* and an active component used in traditional medicine for managing inflammatory respiratory diseases, has been reported to exhibit various biological activities (Akhtar *et al*., 2020; Xu *et al*., 2020; Baharara *et al*., 2021; Zha *et al*., 2023). Hederacoside C has been shown to suppress *Staphylococcus aureus*-induced mastitis via toll-like receptors (Akhtar *et al*., 2020), exhibit chondroprotective effects by inhibiting extracellular matrix degradation (Xu *et al*., 2020), regulate cough and respiratory illnesses (Baharara *et al*., 2021), and ameliorate colitis (Zha *et al*., 2023).

However, there are no reports on the effects of hederacoside C on mucin gene expression in airway epithelial cells and the intracellular signaling pathways involved. Therefore, in this study, we investigate the potential of hederacoside C to influence mRNA expression and glycoprotein production of pulmonary MUC5AC mucin stimulated by EGF in NCI-H292 cells. NCI-H292 cells, a human pulmonary mucoepidermoid cell line, are suitable for studying the intracellular signaling pathways involved in pulmonary mucin gene expression. EGF stimulates the epidermal growth factor receptor (EGFR) signaling pathway, a major regulator of airway mucin production, making it important to examine whether hederacoside C modulates the EGF-induced EGFR-MAPK signaling pathway in airway epithelial NCI-H292 cells (Perrais *et al*., 2002; Shao *et al*., 2003; Evans *et al*., 2009; Le Cras *et al*., 2011; Simoes *et al*., 2019, Chen *et al*., 2021). Investigating whether hederacoside C can modulate the activation of the EGFR-MAPK signaling pathway in response to EGF in NCI-H292 cells could provide insights into its potential therapeutic effects in pulmonary diseases. Understanding how hederacoside C interacts with this signaling pathway could offer valuable insights into its mechanism of action and its potential as a treatment for mucin-related airway diseases.

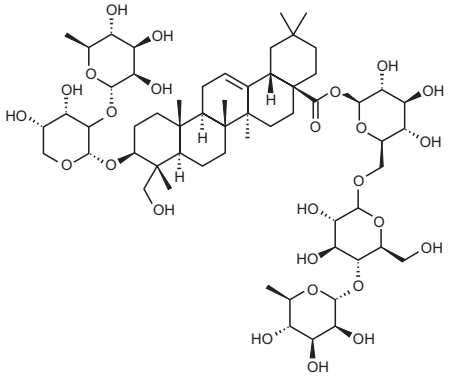

**Fig. 1.** Chemical structure of hederacoside C.

## MATERIALS AND METHODS

### Materials

All the chemicals including hederacoside C (purity: 95.0%) (Fig. 1) used in this experiment were purchased from Sigma-Aldrich (St. Louis, MO, USA) unless otherwise stated. Anti-phospho-EGFR (Y1068) (#3777), anti-phospho-mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) kinase (MEK) 1/2 (S221) (#2338), anti-phospho-p44/42 MAPK (T202/Y204) (#4370), and anti-phospho-p38 MAPK (T180/Y182) (#4511) antibodies were purchased from Cell Signaling Technology Inc. (Danvers, MA, USA). Anti-specificity protein-1 (Sp1) (sc-420) antibody was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Anti-nuclear matrix protein p84 (ab-487) and anti-α-tubulin (ab-471) were purchased from Abcam (Cambridge, MA, USA). Either Goat Anti-rabbit IgG (#401315) or Goat Anti-mouse IgG (#401215) was used as the secondary antibody and purchased from Calbiochem (Carlsbad, CA, USA).

### NCI-H292 cell culture

NCI-H292 cells were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). Cells were cultured in RPMI 1640 supplemented with 10% fetal bovine serum (FBS) in the presence of penicillin (100 units/mL), streptomycin (100 μg/mL) and HEPES (25 mM) at 37°C in a humidified, 5% $CO_2$/95% air, water-jacketed incubator. For serum deprivation, confluent cells were washed twice with phosphate-buffered saline (PBS) and recultured in RPMI 1640 with 0.2% fetal bovine serum for 24 h.

### Treatment of cells with hederacoside C

After 24 h of serum deprivation, cells were pretreated with 1, 5, 10, and 20 μM of hederacoside C for 30 min and then treated with EGF (25 nM) for 24 h in serum-free RPMI 1640. Hederacoside C was dissolved in dimethyl sulfoxide and treated in culture medium (final concentrations of dimethyl sulfoxide were 0.5%). The final pH values of these solutions were between 7.0 and 7.4. Culture medium and 0.5% dimethyl

sulfoxide did not affect mucin gene expression, activity and expression of molecules involved in EGFR-MAPK signaling pathway, in NCI-H292 cells. After 24 h, cells were lysed with buffer solution containing 20 mM Tris, 0.5% NP-40, 250 mM NaCl, 3 mM EDTA, 3 mM EGTA and protease inhibitor cocktail (Roche Diagnostics, IN, USA) and collected to measure the production of MUC5AC glycoprotein (in a 24-well culture plate). The total RNA was extracted in order to measure the expression of MUC5AC mRNA (in a 6-well culture plate) using RT-PCR. For the western blot analysis, cells were treated with hederacoside C for 24 h, followed by EGF treatment for 15 min for upstream signal proteins (EGFR, MEKs, ERKs, and p38 MAPK) and 60 min for Sp1.

### Quantitative analysis of MUC5AC mucin

Airway MUC5AC mucin glycoprotein production was measured using enzyme-linked immunosorbent assay (ELISA). Cell lysates were prepared with PBS at 1:10 dilution, and 100 μL of each sample was incubated at 42°C in a 96-well plate, until it would be dry. Plates were washed three times with PBS and blocked with 2% bovine serum albumin (BSA) (fraction V) for 1 h at room temperature. Plates were washed another three times with PBS and then incubated with 100 μL of 45M1, a mouse monoclonal MUC5AC antibody (1:200) (NeoMarkers, CA, USA), which was diluted with PBS containing 0.05% Tween 20, and dispensed into each well. After 1 h, the wells were washed three times with PBS, and 100 μL of horseradish peroxidase-goat anti-mouse IgG conjugate (1:3,000) was dispensed into each well. After 1 h, plates were washed three times with PBS. Color reaction was developed with 3,3',5,5'-tetramethylbenzidine (TMB) peroxide solution and stopped with 1 N $H_2SO_4$. Absorbance was read at 450 nm.

### Isolation of total RNA and RT-PCR

Total RNA was isolated by using Easy-BLUE Extraction Kit (INTRON Biotechnology, Inc., Seongnam, Korea) and reverse transcribed by using AccuPower RT Premix (BIONEER Corporation, Daejeon, Korea) according to the manufacturer's instructions. Two μg of total RNA was primed with 1 μg of oligo (dT) in a final volume of 50 μL (RT reaction). Two μL of RT reaction product was PCR-amplified in a 25 μL by using Thermorprime Plus DNA Polymerase (ABgene, Rochester, NY, USA). Primers for MUC5AC were (forward) 5′-TGA TCA TCC AGC AGG GCT-3′ and (reverse) 5′-CCG AGC TCA GAG GAC ATA TGG G-3′. Primers for Rig/S15 rRNA, which encodes a small ribosomal subunit protein, a housekeeping gene that was constitutively expressed, were used as quantitative controls. Primers for Rig/S15 were (forward) 5′-TTC CGC AAG TTC ACC TAC C-3′ and (reverse) 5′-CGG GCC GGC CAT GCT TTA CG-3′. The PCR mixture was denatured at 94°C for 2 min followed by 40 cycles at 94°C for 30 s, 60°C for 30 s and 72°C for 45 s. After PCR, 5 μL of PCR products were subjected to 1% agarose gel electrophoresis and visualized with ethidium bromide under a transilluminator.

### Whole cell extract preparation

NCI-H292 cells (confluent in 100 mm culture dish) were pretreated for 24 h at 37°C with 1, 5, 10, and 20 μM of hederacoside C, and then stimulated with EGF (25 nM) for 15 min, in serum-free RPMI 1640. After the treatment of the cells with hederacoside C, media were aspirated, and the cells were washed with cold PBS. For the cell collection, the cells were scraped and centrifuged at 3,000 rpm for 5 min. After the supernatant was discarded, the cell pellet was mixed with RIPA buffer (25 mM Tris-HCl pH 7.6, 150 mM NaCl, 1% NP-40, 1% sodium deoxycholate, 0.1% SDS) for 30 min with continuous agitation. The lysate was centrifuged in a microcentrifuge at 14,000 rpm for 15 min at 4°C. The supernatant was either used, or was immediately stored at −80°C. The amount of protein in extract was quantified by Bradford method.

### Nuclear and cytosolic extracts preparation

After the treatment with hederacoside C as stated, the cells (confluent in 150 mm culture dish) were harvested using Trypsin-EDTA solution and then centrifuged in a microcentrifuge (1,200 rpm, 3 min, 4°C). After the supernatant was discarded, the cell pellet was washed by suspending in PBS. The cytoplasmic and nuclear protein fractions were extracted using NE-PER® nuclear and cytoplasmic extraction reagent (Thermo-Pierce Scientific, Waltham, MA, USA) according to the manufacturer's instructions. Both extracts were stored at −20°C. The amount of protein in extracts was quantified by Bradford method.

### Western blotting for the detection of proteins

Whole cell, cytosolic, and nuclear extracts containing proteins (each 50 μg as proteins) were subjected to 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred onto the polyvinylidene difluoride (PVDF) membrane. The blots were blocked using 5% skim milk and probed with appropriate primary antibody in blocking buffer overnight at 4°C. The membrane was washed with PBS and then probed with the secondary antibody conjugated with horseradish peroxidase. Immunoreactive bands were detected by an enhanced chemiluminescence kit (Pierce ECL western blotting substrate, Thermo Scientific, Waltham, MA, USA) (Kim *et al*., 2023b; Lee *et al*., 2023a; Yoon *et al*., 2023).

### Computational analysis

The protein structures of EGFR, MEK1/2, ERK1/2, and p38 MAPK were determined using the Swiss model. Protein templates were selected based on BLAST analysis using the NCBI BLAST program, and protein sequences were retrieved from UniProt (https://www.uniprot.org/) (Uniprot consortium, 2023). The homology models were validated using PROCHECK. Molecular docking studies were conducted to examine the binding interactions of hederacoside C with these specific proteins. The 3D structure of hederacoside C was obtained in pdf file format from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/, accessed 04 Jan 2024) (Kim *et al*., 2023d). All ligands' internal energies were optimized using the Chem3D Pro 12.0 computational program (CambridgeSoft Corporation, Cambridge, MA, USA). Molecular docking results provided insights into the degree of ligand interaction with the active sites of the macromolecules. The active binding sites of the selected proteins were investigated within the initial protein grids (40×40×40) using PyMOL, AutoDock Vina, and Drug Discovery Studio (v.20.1.0.19295) (Morris *et al*., 2009; Trott and Olson, 2010).

### Statistics

The means of individual groups were converted to percent control and expressed as mean ± SEM. The difference between groups was assessed using a one-way ANOVA and the

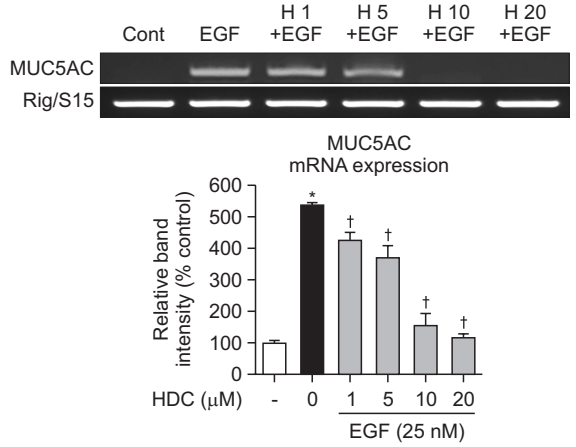


**Fig. 2.** Effect of hederacoside C on EGF-induced MUC5AC mucin mRNA expression from NCI-H292 cells. NCI-H292 cells were pretreated with varying concentrations of hederacoside C for 30 min and then stimulated with EGF (25 nM), for 24 h. Cell lysates were collected for measurement of MUC5AC mucin mRNA expression using RT-PCR. Three independent experiments were performed, and the representative data were shown. *Significantly different from control ($p$<0.05). †Significantly different from EGF alone ($p$<0.05). cont: control, H, HDC: hederacoside C, concentration unit is μM.

Holm-Sidak test as a post-hoc test. A $p$-value of <0.05 was considered significantly different.

## RESULTS

### Effect of hederacoside C on EGF-induced MUC5AC mRNA expression and glycoprotein production of MUC5AC mucin

MUC5AC mRNA expression induced by EGF was inhibited by pretreatment with hederacoside C (Fig. 2). Hederacoside C also suppressed EGF-induced MUC5AC mucin glycoprotein biosynthesis (production). The amounts of MUC5AC mucin in the cells of hederacoside C-treated cultures were 100 ± 5% (control), 341 ± 11% (25 nM of EGF alone), 304 ± 11% (EGF plus hederacoside C 1 μM), 300 ± 16% (EGF plus hederacoside C 5 μM), 222 ± 14% (EGF plus hederacoside C 10 μM), and 194 ± 28% (EGF plus hederacoside C 20 μM), respectively (Fig. 3). Cytotoxicity was checked by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and there was no cytotoxic effect of hederacoside C, at 1, 5, 10, or 20 μM concentration (data not shown).

### Effect of hederacoside C on the phosphorylation of EGFR

EGFR signaling cascade (Ras/Raf/MAPK) is recognized to be one of the major regulatory pathways for MUC5AC mucin production. EGF (25 nM, 24 h) stimulated the phosphorylation of EGFR, whereas hederacoside C inhibited EGF-stimulated phosphorylation of EGFR, as can be seen by western blot analysis in Fig. 4.

### Effect of hederacoside C on the phosphorylation MAPK signaling proteins

To elucidate the effect of hederacoside C on the downstream signaling, we investigated a potential activity of hederacoside C on MEK1/2, ERK1/2, and p38 MAPK pathway, which are directly involved into MUC5AC mucin gene expression. EGF stimulated the phosphorylation of MEK1/2, whereas hederacoside C suppressed the phosphorylation MEK1/2, in NCI-H292 cells. EGF stimulated the phosphorylations of p44/42 and p38, whereas hederacoside C suppressed the phosphorylations of p44/42 (ERK1/2) and p38 MAPK, as shown by western blot analysis (Fig. 5).

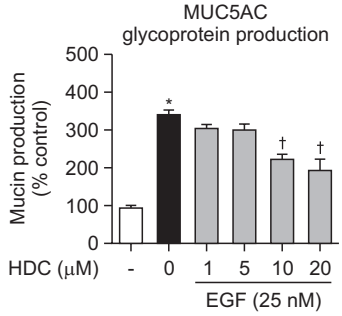


**Fig. 3.** Effect of hederacoside C on EGF-induced MUC5AC mucin glycoprotein production from NCI-H292 cells. NCI-H292 cells were pretreated with varying concentrations of hederacoside C for 30 min and then stimulated with EGF (25 nM), for 24 h. Cell lysates were collected for measurement of MUC5AC mucin glycoprotein production by ELISA. Each bar represents a mean ± SEM of 3 culture wells compared to the control set at 100%. Three independent experiments were performed, and the representative data were shown. *Significantly different from control ($p$<0.05). †Significantly different from EGF alone ($p$<0.05). cont: control, HDC: hederacoside C, concentration unit is μM.

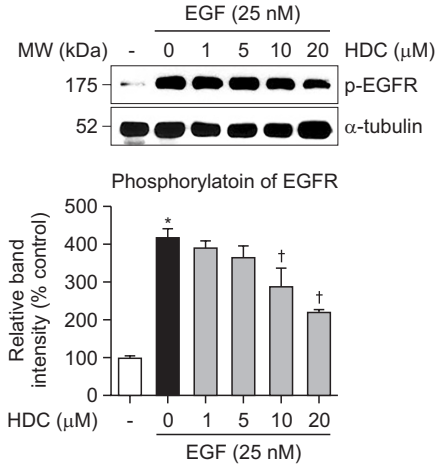


**Fig. 4.** Effect of hederacoside C on the phosphorylation of EGFR in NCI-H292 cells. NCI-H292 cells were pretreated with varying concentrations of hederacoside C for 24 h and then stimulated with epidermal growth factor (EGF) (25 nM) for 15 min. Whole cell extract was collected and western blot analysis of the cellular proteins with anti-phospho-EGFR (Y1068) antibody was conducted. Three independent experiments were performed and the representative data were shown. *Significantly different from control ($p$<0.05). †Significantly different from EGF alone ($p$<0.05). cont: control, HDC: hederacoside C, EGFR: EGF receptor, concentration unit is μM.

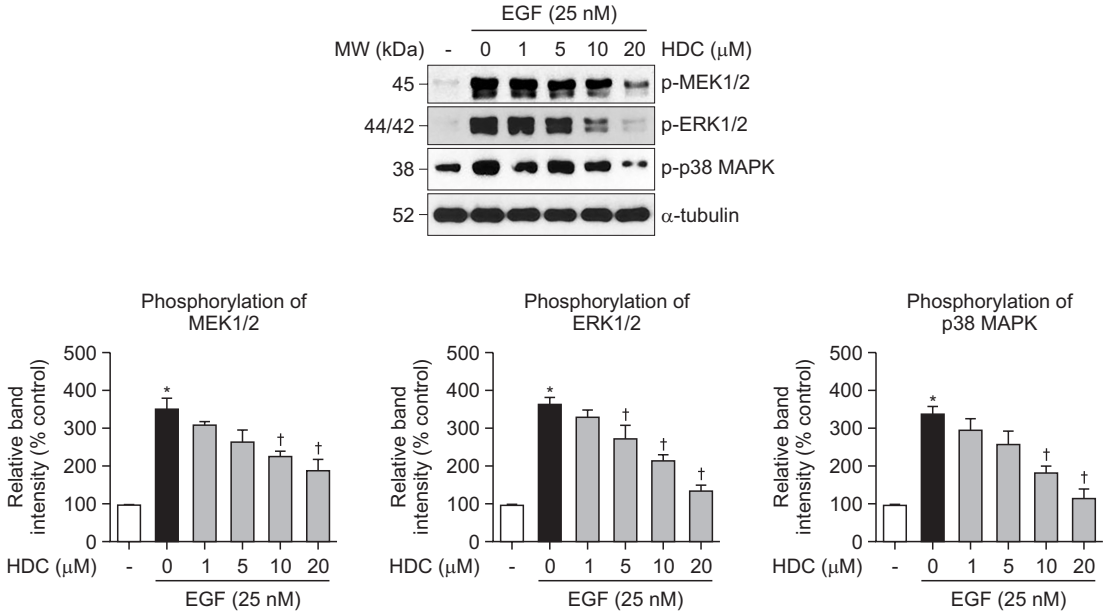


**Fig. 5.** Effect of hederacoside C on the phosphorylation of MEK1/2, the phosphorylation of p44/42, and the phosphorylation of p38, in NCI-H292 cells. NCI-H292 cells were pretreated with varying concentrations of hederacoside C for 24 h and then stimulated with epidermal growth factor (EGF) (25 nM) for 15 min. Whole cell extract was collected and western blot analysis of the cellular proteins with anti-phospho-MEK1/2 (Ser221), anti-phospho-p44/42 (Thr202/Tyr204), and anti-phospho-p38 (Thr180/Tyr182) antibodies was conducted. Three independent experiments were performed and the representative data were shown. *Significantly different from control ($p$<0.05). †Significantly different from EGF alone ($p$<0.05). cont: control, HDC: hederacoside C, concentration unit is μM.

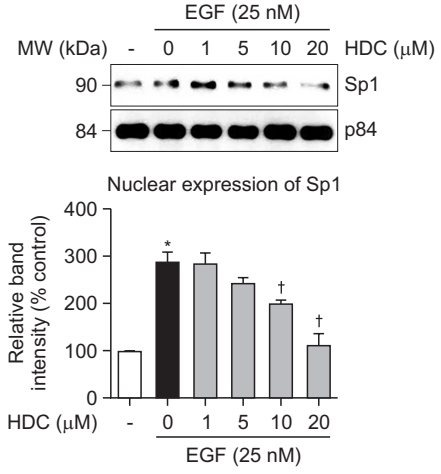


**Fig. 6.** Effect of hederacoside C on the nuclear expression of Sp1 in NCI-H292 cells. NCI-H292 cells were pretreated with varying concentrations of hederacoside C for 24 h and then stimulated with epidermal growth factor (EGF) (25 nM) for 60 min. The nuclear protein extracts were prepared and subjected to western blot analysis using antibody against Sp1. As a loading control, p84 levels were analyzed. Three independent experiments were performed and the representative data were shown. *Significantly different from control ($p$<0.05). †Significantly different from EGF alone ($p$<0.05). cont: control, HDC: hederacoside C, Sp1: specificity protein-1, concentration unit is μM.

### Effect of hederacoside C on the nuclear expression of Sp1

As a transcription factor from Sp family, Sp1 is an essential mediator in EGF-stimulated MUC5AC mucin gene expression. After activation of ERK signal, EGF upregulated nuclear expression of Sp1 and this transcriptional factor binds to DNA interacting site for MUC5AC mucin gene expression. In the present study, EGF stimulated the nuclear expression of Sp1, whereas hederacoside C suppressed the nuclear expression of Sp1 (Fig. 6). This, in turn, led to the down-regulation of the production of MUC5AC mucin glycoprotein, in NCI-H292 cells.

### Molecular interactions of hederacoside C with EGFR and MAPK proteins

Homology models of EGFR (UniProt accession ID: P00533), MEK1 (UniProt accession ID: Q13233), MEK2 (UniProt accession ID: P36507), ERK1 (UniProt accession ID: P27361), ERK2 (UniProt accession ID: P28482), and p38 MAPK (UniProt accession ID: Q16644) were generated using the Swiss model. Molecular docking studies revealed that hederacoside C exhibited strong binding interactions with EGFR, MEK1/MEK2, ERK1/ERK2, and p38 MAPK proteins, with binding affinities ranging from –7.4 to –10.3 kcal/mol (Fig. 7). Among these interactions, hederacoside C showed the highest binding affinity for MEK1 (–10.3 kcal/mol; Fig. 7B) and the lowest for ERK1 (–7.4 kcal/mol; Fig. 7D). Additionally, hederacoside C exhibited moderate interactions with the EGFR (–8.3 kcal/mol), MEK2 (–9.3 kcal/mol), ERK2 (–9.6 kcal/mol), and p38 MAPK (–8.5 kcal/mol), respectively (Fig. 7A, 7C, 7E, 7F). These results suggest that hederacoside C suppresses MUC5AC gene expression by inhibiting the activation of EGFR.

## DISCUSSION

In current clinical practice, various agents, including glu-

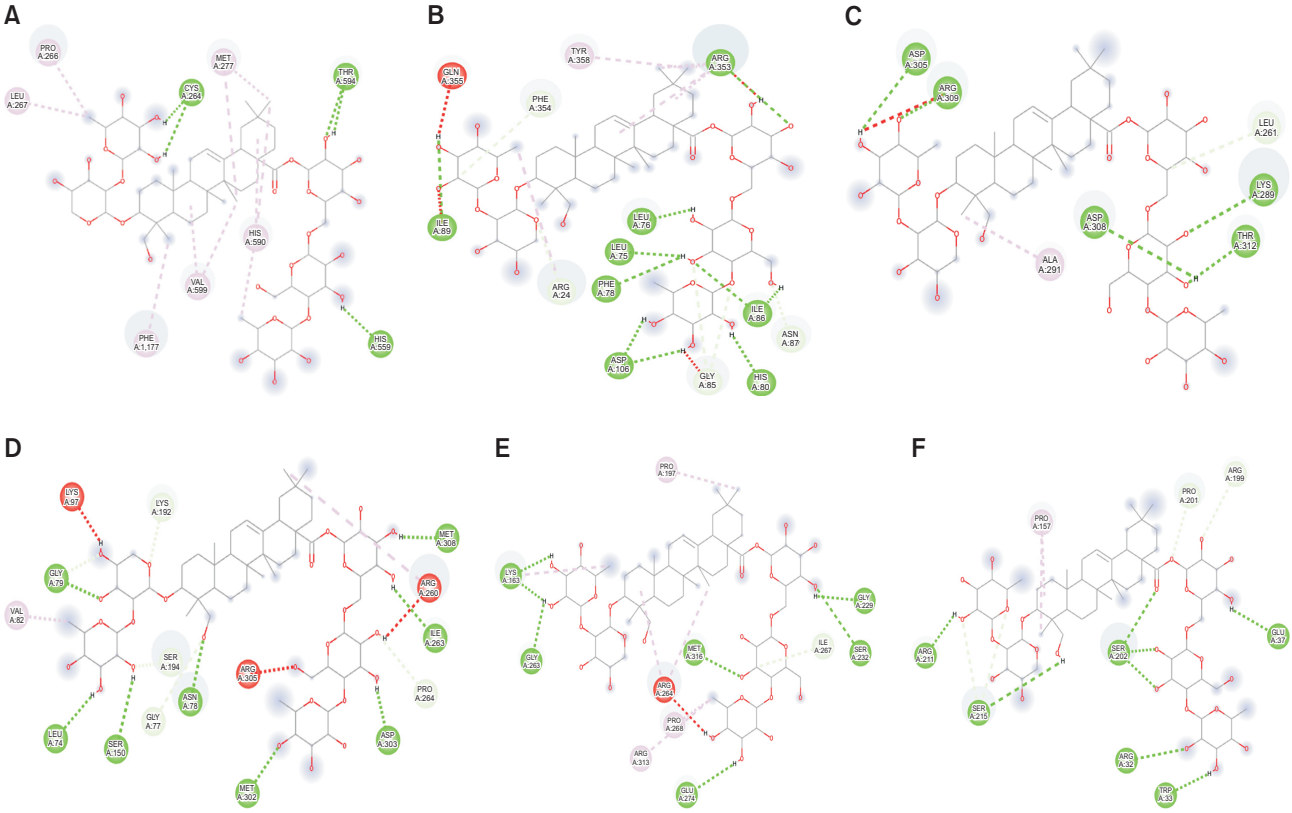


**Fig. 7.** Molecular interactions of hederacoside C with EGFR and MAP kinase proteins. The 2D interaction diagram depicts hederacoside C's interactions with (A) EGFR, (B) MEK1, (C) MEK2, (D) ERK1, (E) ERK2, and (F) p38 MAPK. Molecular docking studies revealed that hederacoside C exhibited strong binding interactions with EGFR, MEK1/MEK2, ERK1/ERK2, and p38 MAPK proteins, with binding affinities ranging from –7.4 to –10.3 kcal/mol (Fig. 7). Among these interactions, hederacoside C showed the highest binding affinity for MEK1 (–10.3 kcal/mol; Fig. 7B) and the lowest for ERK1 (–7.4 kcal/mol; Fig. 7D). Additionally, hederacoside C exhibited moderate interactions with the EGFR (–8.3 kcal/mol), MEK2 (–9.3 kcal/mol), ERK2 (–9.6 kcal/mol), and p38 MAPK (–8.5 kcal/mol), respectively (Fig. 7A, C, E, F). These results suggest that hederacoside C suppresses MUC5AC gene expression by inhibiting the activation of EGFR.

cocorticoids, are used to manage pulmonary diseases associated with airway mucus hypersecretion. However, these treatments often fail to deliver significant clinical benefits and may result in various side effects (Li *et al*., 2020). To date, no specific pharmacological agent effectively regulates the production and/or secretion of airway MUC5AC mucin. The development of such an agent is urgently needed to control the hyperproduction and/or hypersecretion of pathologically altered mucus in pulmonary diseases. Effective management of inflammatory pulmonary diseases requires the regulation of the inflammatory response.

The present study demonstrates that hederacoside C, a natural product with anti-inflammatory properties, inhibits the production of MUC5AC mucin glycoprotein and the expression of MUC5AC mucin mRNA induced by epidermal growth factor (EGF) (Fig. 2, 3). These findings suggest that hederacoside C can directly influence airway epithelial cells by regulating mucin gene expression at both the transcriptional and translational levels. It remains unclear whether hederacoside C influences the production and secretion of MUC5AC mucin glycoprotein based on the findings of this study. Further research is needed to clarify this.

Several research groups have provided a comprehensive overview of the EGFR signaling pathway and its role in regulating MUC5AC mucin gene expression. EGF has been shown to induce MUC5AC mucin gene expression by activating the EGFR signaling pathway in NCI-H292 cells. EGFR is upregulated in asthmatic airways and is a key regulator of epithelial function, transducing extracellular signals into intracellular signaling cascades, such as the MEK-MAPK pathway. Activated MAPK, in turn, activates the Sp1 transcription factor, which initiates the expression of the MUC5AC mucin gene (Lemmon and Schlessinger, 1994; Burgel *et al*., 2001). MAPKs, particularly MEK, ERK, and p38 MAPK, are critical kinases involved in regulating pro-inflammatory chemokines and the production of cellular macrophages (Bondeson, 1997). Numerous studies have shown that EGF induces phosphorylation of MEK1/2, ERK1/2 (p44/42 MAPK), and p38 MAPK, which leads to MUC5AC gene expression (Rescan *et al*., 2005; Tashiro *et al*., 2016; Zhao *et al*., 2016). The EGFR-MEK-MAPK-Sp1 signaling cascade plays a significant role in MUC5AC gene expression (Hewson *et al*., 2004). Inhibitors of EGFR tyrosine kinase can suppress EGF-stimulated MUC5AC gene expression, indicating that hyperproduction of MUC5AC mucin is driven by EGFR pathway activation (Perrais *et al*., 2002; Mata *et al*., 2005; Tang *et al*., 2018). Sp1 is a well-characterized transcription factor involved in the transcription of various genes, including MUC5AC (Zhang *et al*., 2023).

In this study, the data show that hederacoside C induces EGFR phosphorylation in airway epithelial cells (Fig. 4). Additionally, hederacoside C inhibits EGF-mediated phosphorylation of MEK, ERK, and p38 MAPK (Fig. 5). Finally, pretreatment of NCI-H292 cells with hederacoside C blocks EGF-induced nuclear expression of Sp1, as shown in Fig. 6. These results suggest that the inhibitory effect on the MAPK pathway plays a partial role in mediating hederacoside C's regulatory effect on MUC5AC glycoprotein production. Collectively, these findings indicate that hederacoside C influences MUC5AC gene expression by regulating the EGFR-MAPK-Sp1 signaling pathway. Moreover, computational analyses reveal that hederacoside C has significant binding interactions with EGFR, MEK2, ERK1, and p38 MAPK proteins, providing mechanistic insights into its regulation of EGFR signaling (Fig. 7).

Taken together, while current treatments for airway mucus hypersecretion have limited efficacy and cause side effects, hederacoside C holds promise as a potential therapeutic agent due to its ability to suppress MUC5AC mucin gene expression. This suggests its potential for further preclinical and clinical development in managing inflammatory pulmonary diseases. Further research and clinical trials are necessary to validate the efficacy and safety of hederacoside C in regulating mucin production and secretion, paving the way for its potential use in clinical settings.

## CONFLICT OF INTEREST

The authors have declared that there is no conflict of interest.

## REFERENCES


Abrami, M., Biasin, A., Tescione, F., Tierno, D., Dapas, B., Carbone, A., Grassi, G., Conese, M., Di Gioia, S., Larobina, D. and Grassi, M. (2024) Mucus structure, viscoelastic properties, and composition in chronic respiratory diseases. *Int. J. Mol. Sci.* **25**, 1933.

Akhtar, M., Shaukat, A., Zahoor, A., Chen, Y., Wang, Y., Yang, M., Umar, T., Guo, M. and Deng, G. (2020) Hederacoside-C inhibition of Staphylococcus aureus-induced mastitis via TLR2 & TLR4 and their downstream signaling NF-kappaB and MAPKs pathways *in vivo* and *in vitro*. *Inflammation* **43**, 579-594.

Allinson, J. P., Hardy, R., Donaldson, G. C., Shaheen, S. O., Kuh, D. and Wedzicha, J. A. (2016) The presence of chronic mucus hypersecretion across adult life in relation to chronic obstructive pulmonary disease development. *Am. J. Respir. Crit. Care Med.* **193**, 662-672.

Baharara, H., Moghadam, A. T., Sahebkar, A., Emami, S. A., Tayebi, T. and Mohammadpour, A. H. (2021) The effects of ivy (Hedera helix) on respiratory problems and cough in humans: a review. *Adv. Exp. Med. Biol.* **1328**, 361-376.

Bondeson, J. (1997) The mechanisms of action of disease-modifying antirheumatic drugs: a review with emphasis on macrophage signal transduction and the induction of proinflammatory cytokines. *Gen. Pharmacol.* **29**, 127-150.

Burgel, P. R., Lazarus, S. C., Tam, D. C., Ueki, I. F., Atabai, K., Birch, M. and Nadel, J. A. (2001) Human eosinophils induce mucin production in airway epithelial cells via epidermal growth factor receptor activation. *J. Immunol.* **167**, 5948-5954.

Chen, X., Yang, J., Shen, H., Zhang, X., Wang, H., Wu, G., Qi, Y., Wang, L. and Xu, W. (2021) Muc5ac production inhibited by decreased lncRNA H19 via PI3K/Akt/NF-kB in asthma. *J. Asthma Allergy* **14**, 1033-1043.

Evans, C. M., Kim, K., Tuvim, M. J. and Dickey, B. F. (2009) Mucus hypersecretion in asthma: causes and effects. *Curr. Opin. Pulm. Med.* **15**, 4-11.

Evans, C. M. and Koo, J. S. (2009) Airway mucus: the good, the bad, the sticky. *Pharmacol. Ther.* **121**, 332-348.

Hewson, C. A., Edbrooke, M. R. and Johnston, S. L. (2004) PMA induces the MUC5AC respiratory mucin in human bronchial epithelial cells via PKC, EGF/TGF-alpha, Ras/Raf, MEK, REK and Sp1-dependent mechanisms. *J. Mol. Biol.* **344**, 683-695.

Hossain, R., Kim, K. I., Jin, F., Lee, H. J. and Lee, C. J. (2022a) Betulin, an anti-inflammatory triterpenoid compound, regulates MUC5AC mucin gene expression through NF-kB signaling in human airway epithelial cells. *Biomol. Ther. (Seoul)* **30**, 540-545.

Hossain, R., Kim, K. I., Li, X., Lee, H. J. and Lee, C. J. (2022b) Involvement of IKK/IkB$\alpha$/NF-kB p65 signaling into the regulative effect of engeletin on MUC5AC mucin gene expression in human airway epithelial cells. *Biomol. Ther. (Seoul)* **30**, 473-478.

Huh, E., Choi, J. G., Choi, Y., Ju, I. G., Noh, D., Shin, D. Y., Kim, D. H., Park, H. J. and Oh, M. S. (2023) 6-Shogaol, an active ingredient of ginger, improves intestinal and brain abnormalities in proteus mirabilis-induced Parkinson's disease mouse model. *Biomol. Ther. (Seoul)* **31**, 417-424.

Hwang, S. Y., Wi, K., Yoon, G., Lee, C. J., Lee, S. I., Jung, J. G., Jeong, H. W., Kim, J. S., Choi, C. H., Na, C. S., Shim, J. H. and Lee, M. H. (2023) Licochalcone D inhibits skin epidermal cells transformation through the regulation of AKT signaling pathways. *Biomol. Ther. (Seoul)* **31**, 682-691.

Jang, J. H., Yang, G., Seok, J. K., Kang, H. C., Cho, Y. Y., Lee, H. S. and Lee, J. Y. (2023) Loganin prevents hepatic steatosis by blocking NLRP3 inflammasome activation. *Biomol. Ther. (Seoul)* **31**, 40-47.

Kim, E. J., Yoon, Y. P., Woo, K. W., Kim, J. H., Min, S. Y., Lee, H. J., Lee, S. K., Hong, J. H., Lee, K. R. and Lee, C. J. (2016) Verticine, ebeiedine and suchengbeisine isolated from the bulbs of Fritillaria thunbergiiMiq. inhibited the gene expression and production of MUC5AC mucin from human airway epithelial cells. *Phytomedicine* **23**, 95-104.

Kim, J. O., Sikder, M. A., Lee, H. J., Rahman, M., Kim, J. H., Chang, G. T. and Lee, C. J. (2012) Phorbol ester or epidermal growth factor-induced MUC5AC mucin gene expression and production from airway epithelial cells are inhibited by pyronaridine and wogonin. *Phytother. Res.* **26**, 1784-1788.

Kim, K. I., Hossain, R., Ryu, J., Lee, H. J. and Lee, C. J. (2023a) Regulation of the gene expression of airway MUC5AC mucin through NF-$\kappa$B signaling pathway by artesunate, an antimalarial agent. *Biomol. Ther. (Seoul)* **31**, 544-549.

Kim, N. H. and Lee, A. Y. (2023b) Growth factors upregulated by uric acid affect guanine deaminase-induced melanogenesis. *Biomol. Ther. (Seoul)* **31**, 89-96.

Kim, N., Kwon, J., Shin, U. S. and Jung, J. (2023c) stimulatory anticancer effect of resveratrol mediated by G protein-coupled estrogen receptor in colorectal cancer. *Biomol. Ther. (Seoul)* **31**, 655-660.

Kim, S., Chen, J., Cheng, T., Gindulyte, A., He, J., He, S., Li, Q., Shoemaker, B. A., Thiessen, P. A., Yu, B., Zaslavsky, L., Zhang, J. and Bolton, E. E. (2023d) PubChem 2023 update. *Nucleic Acids Res.* **51**, D1373-D1380.

Ko, H., An, S., Jang, H., Ahn, S., Park, I. G., Hwang, S. Y., Gong, J., Oh, S., Kwak, S. Y., Choi, W. J., Kim, H. and Noh, M. (2023) Macakurzin C derivatives as a novel pharmacophore for pan-peroxisome proliferator-activated receptor modulator. *Biomol. Ther. (Seoul)* **31**, 312-318.

Le Cras, T. D., Acciani, T. H., Mushaben, E. M., Kramer, E. L., Pastura, P. A., Hardie, W. D., Korfhagen, T. R., Sivaprasad, U., Ericksen, M., Gibson, A. M. and Holtzman, M. J. (2011) Epithelial EGF receptor signaling mediates airway hyperreactivity and remodeling in a mouse model of chronic asthma. *Am. J. Physiol. Lung Cell Mol. Physiol.* **300**, L414-L421.

Lee, C., Jang, J. H. and Park, G. H. (2023a) $\alpha$-Pinene attenuates methamphetamine-induced conditioned place preference in C57BL/6 mice. *Biomol. Ther. (Seoul)* **31**, 411-416.

Lee, H. J., Park, J. S., Yoon, Y. P., Shin, Y. J., Lee, S. K., Kim, Y. S., Hong, J. H., Son, K. H. and Lee, C. J. (2015) Dioscin and methylprotodioscin isolated from the root of Asparagus cochinchinensis

suppressed the gene expression and production of airway MUC5AC mucin induced by phorbol ester and growth factor. *Phytomedicine* **22**, 568-572

Lee, S. O., Lee, M. H., Kwak, A. W., Lee, J. Y., Yoon, G., Joo, S. H., Choi, Y. H., Park, J. W. and Shim, J. H. (2023b) Licochalcone H targets EGFR and AKT to suppress the growth of oxaliplatin -sensitive and -resistant colorectal cancer cells. *Biomol. Ther. (Seoul)* **31**, 661-673.

Lemmon, M. A. and Schlessinger, J. (1994) Regulation of signal transduction and signal diversity by receptor oligomerization. *Trends Biochem. Sci.* **19**, 459-463.

Li, X., Jin, F., Lee, H. J. and Lee, C. J. (2020) Recent advances in the development of novel drug candidates for regulating the secretion of pulmonary mucus. *Biomol. Ther. (Seoul)* **28**, 293-301.

Mann, T. S., Larcombe, A. N., Wang, K. C., Shamsuddin, D., Landwehr, K. R., Noble, P. B. and Henry, P. J. (2022) Azithromycin inhibits mucin secretion, mucous metaplasia, airway inflammation and airways hyperresponsiveness in mice exposed to house dust mite extract. *Am. J. Physiol. Lung Cell Mol. Physiol.* **322**, L683-L698.

Mata, M., Sarria, B., Buenestado, A., Cortijo, J., Cerda, M. and Morcillo, E. J. (2005) Phosphodiesterase 4 inhibition decreases MUC5AC expression induced by epidermal growth factor in human airway epithelial cells. *Thorax* **60**, 144-152.

Morris, G. M., Huey, R., Lindstrom, W., Sanner, M. F., Belew, R. K., Goodsell, D. S. and Olson, A. J. (2009) AutoDock4 and AutoDockTools4: automated docking with selective receptor flexibility. *J. Comput. Chem.* **30**, 2785-2791.

Perrais, M., Pigny, P., Copin, M. C., Aubert, J. P. and Van Seuningen, I. (2002) Induction of MUC2 and MUC5AC mucins by factors of the epidermal growth factor (EGF) family is mediated by EGF receptor/Ras/Raf/extracellular signal-regulated kinase cascade and Sp1. *J. Biol. Chem.* **277**, 32258-32267.

Rescan, C., Le Bras, S., Lefebvre, V. H., Frandsen, U., Klein, T., Foschi, M., Pipeleers, D. G., Scharfmann, R., Madsen, O. D. and Heimberg, H. (2005) EGF-induced proliferation of adult human pancreatic duct cells is mediated by the MEK/ERK cascade. *Lab. Invest.* **85**, 65-74.

Ryu, J., Kim, K. I., Hossain, R., Lee, M., Hong, J. T., Lee, H. J. and Lee, C. J. (2023) Meclofenamate suppresses MUC5AC mucin gene expression by regulating the NF-kB signaling pathway in human pulmonary mucoepidermoid NCI-H292 cells. *Biomol. Ther. (Seoul)* **31**, 306-311.

Ryu, J., Lee, H. J., Park, S. H., Sikder, M. A., Kim, J. O., Hong, J. H., Seok, J. H. and Lee, C. J. (2013) Effect of prunetin on TNF-α-induced MUC5AC mucin gene expression, production, degradation of IκB and translocation of NF-κB p65 in human airway epithelial cells. *Tuberc. Respir. Dis. (Seoul)* **75**, 205-209.

Ryu, J., Lee, H. J., Park, S. H., Kim, J., Lee, D., Lee, S. K., Kim, Y. S., Hong, J. H., Seok, J. H. and Lee, C. J. (2014) Effects of the root of Platycodon grandiflorum on airway mucin hypersecretion *in vivo* and platycodinD(3) and deapi-platycodin on production and secretion of airway mucin *in vitro*. *Phytomedicine* **21**, 529-533.

Seo, H. S., Sikder, M. A., Lee, H. J., Ryu, J. and Lee, C. J. (2014) Apigenin inhibits tumor necrosis factor-α-induced production and gene expression of mucin through regulating nuclear factor-kappa b signaling pathway in airway epithelial cells. *Biomol. Ther. (Seoul)* **22**, 525-531.

Shah, B. K., Singh, B., Wang, Y., Xie, S. and Wang, C. (2023) Mucus hypersecretion in chronic obstructive pulmonary disease and its treatment. *Mediators Inflamm.* **2023**, 8840594.

Shao, M. X., Ueki, I. F. and Nadel, J. A. (2003) TNF-alpha converting enzyme mediated MUC5AC mucin expression in cultured human airway epithelial cells. *Proc. Natl. Acad. Sci. U. S. A.* **100**, 11618-11623.

Sikder, M. A., Lee, H. J., Mia, M. Z., Park, S. H., Ryu, J., Kim, J. H., Min, S. Y., Hong, J. H., Seok, J. H. and Lee, C. J. (2014) Inhibition of TNF-α-induced MUC5AC mucin gene expression and production by wogonin through the inactivation of NF-κB signaling in airway epithelial cells. *Phytother. Res.* **28**, 62-68.

Simoes, F. B., Quaresma, M. C., Clarke, L. A., Silva, I. A., Pankonien, I., Railean, V., Kmit, A. and Amaral, M. D. (2019) TMEM16A chloride channel does not drive mucus production. *Life Sci. Alliance* **2**, e201900462.

Tang, L., Chen, Q., Sun, L., Zhu, L., Liu, J., Meng, Z., Ni, Z. and Wang, X. (2018) Curcumin suppresses MUC5AC production via interfering with the EGFR signaling pathway. *Int. J. Mol. Med.* **42**, 497-504.

Tashiro, E., Henmi, S., Odake, H., Ino, S. and Imoto, M. (2016) Involvement of the MEK/ERK pathway in EGF-induced E-cadherin down-regulation. *Biochem. Biophys. Res. Commun.* **477**, 801-806.

Trott, O. and Olson, A. J. (2010) AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J. Comput. Chem.* **31**, 455-461.

UniProt Consortium (2023) UniProt: the universal protein knowledgebase in 2023. *Nucleic Acids Res.* **51**, D523-D531.

Vestbo, J. and Hogg, J. C. (2006) Convergence of the epidemiology and pathology of COPD. *Thorax* **61**, 86-88.

Xu, H. C., Wu, B., Ma, Y. M., Xu, H., Shen, Z. H. and Chen, S. (2020) Hederacoside-C protects against AGEs-induced ECM degradation in mice chondrocytes. *Int. Immunopharmacol.* **84**, 106579.

Yoon, H. J., Kang, D. H., Jin, F., Bang, J. S., Sohn, U. D. and Je, H. D. (2023) The effect of luteolin on the modulation of vascular contractility via ROCK and CPI-17 inactivation. *Biomol. Ther. (Seoul)* **31**, 193-199.

Zha, Z. X., Lin, Y., Wang, K. X., Zhang, Y. L., Li, D., Xu, G. Q., Xu, Q. M. and Liu, Y. L. (2023) Hederacoside C ameliorates colitis via restoring impaired intestinal barrier through moderating S100A9/MAPK and neutrophil recruitment inactivation. *Acta Pharmacol. Sin.* **44**, 105-119.

Zhang, J., He, W., Hu, X., Fang, X., Wang, G., Tang, R., Zhang, P. and Li, Q. (2023) Molecular insight of p53/Sp1/MUC5AC axis in the tumorigenesis and progression of lung adenocarcinoma. *Clin. Exp. Pharmacol. Physiol.* **50**, 28-38.

Zhao, Y., Feresin, R. G., Falcon-Perez, J. M. and Salazar, G. (2016) Differential targeting of SLC30A10/ZnT10 heterodimers to endolysosomal compartments modulates EGF-induced MEK/ERK1/2 activity. *Traffic* **17**, 267-288.