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Imperatae Rhizoma extract alleviates atopic dermatitis through regulating PI3K/AKT/NF- κ B signaling pathway and *Staphylococcus aureus*

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ABSTRACT

Imperatae Rhizoma belongs to the Poaceae family and is the rhizome of the perennial plant *Imperata cylindrica*. As a versatile traditional Chinese medicinal herb that is both edible and medicinal, it is used to treat hematuria, damp-heat jaundice, vomiting, and bleeding. Atopic dermatitis (AD) is an inflammatory dermatitis characterized by chronic, recurrent, pruritic conditions. However, the adverse effects of current mainstream therapies include commonly used medications seriously affect the quality of patients' life. This study investigates the role of Imperatae Rhizoma extract (IRE) in AD and underlying mechanisms. Using a dinitrochlorobenzene-induced AD mouse model, we found that IRE alleviates AD symptoms by reducing skin dryness, itching, and ear swelling, and modulating epidermal thickening and mast cell infiltration. The liquid chromatography-mass spectrometry combined with the self-built database identification revealed 14 components in IRE, primarily consisting of phytosterols and triterpenes. Network pharmacology suggests IRE's anti-inflammatory effects may involve the phosphatidylinositol 3-kinase/protein kinase B/nuclear factor- κ B (PI3K/AKT/NF- κ B) pathway. *In vitro*, IRE mitigated the activation of this pathway, inhibiting NO and inducible nitric oxide synthase (iNOS) expression, and reducing tumor necrosis factor- α and interleukin-6 release. IRE also enhanced human immortalized keratinocyte cell line HaCaT cell migration, promoting skin healing, and showed inhibitory effects on *Staphylococcus aureus* on the surface of skin. In conclusion, IRE may serve as a potential treatment for AD by reducing inflammation through the PI3K/AKT/NF- κ B pathway and regulating skin microbiota. This research not only provides a theoretical basis for the application of IRE in the treatment of AD, but also offers a new direction for the development of food and medicine homologous natural compound-based therapies in dermatology.

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1. Introduction

Atopic dermatitis (AD) is an inflammatory dermatitis characterized by chronic, recurrent, pruritic conditions. The pathogenesis of AD is complex and is thought to be related to skin barrier dysfunction, a combination of genetic and environmental factors, and skin microbial

interactions^[1]. Skin inflammation is one of the important signs of AD. Patients with AD have elevated levels of the inflammatory factors tumor necrosis factor- α (TNF- α), interleukin-6 (IL-6), and interleukin-1 β (IL-1 β) in the serum, which are correlated with the severity of AD. According to published research, macrophages can build up in either acute or chronic inflammatory skin, which can exacerbate AD by promoting the expression of inflammatory proteins and cytokine production^[2]. It is now suggested that the pathology of AD involves a balance between T helper 1 cells (Th1) and T helper 2 cells (Th2) immune responses, and that the imbalanced Th2 response

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in AD patients leads to increased inflammatory factors production, thus promoting AD symptoms. Current mainstream therapies include commonly used medications such as topical corticosteroids (TCSs), calcium phosphatase inhibitors, antihistamines, antibiotics, as well as systemic immunosuppression and phototherapy^[3-4]. However, the adverse effects of drugs affect the quality of patients' life seriously, for example, glucocorticoids can cause skin atrophy, capillary dilation, rosacea, glaucoma, hyperglycemia, and hypertension. Therefore, finding natural products that are both safe and effective is crucial for AD therapy and prognosis^[5-6].

Imperatae Rhizoma belongs to the Poaceae family and is the rhizome of the perennial plant *Imperata cylindrica*. It is widely distributed in southwestern Asia, mainly in tropical and subtropical regions. As a versatile traditional Chinese medicinal herb that is both edible and medicinal, *Imperatae Rhizoma* can be used both orally and topically and is considered a safe and effective therapeutic strategy. In traditional Chinese medicine, *Imperatae Rhizoma* can be used alone or in combination with other herbal medicines to treat hematuria, damp-heat jaundice, vomiting, and bleeding^[7]. Modern pharmacological studies have shown that saponins, flavonoids, phenolic compounds, and glycosides are its main active ingredients, with pharmacological activities including immune regulation, antibacterial effects, anti-tumor effects, liver protection, and treatment of hypertension^[8-9]. There have been some studies on the anti-inflammatory activity of *Imperatae Rhizoma* extract (IRE), but there is a lack of research on its role and mechanism in improving AD.

Phosphatidylinositol 3-kinase/protein kinase B (PI3K/AKT) is an important signaling pathway that regulates cell physiological activities^[10-11]. Several illnesses, including psoriasis, has been shown to affect the inflammatory response. Several investigations have also demonstrated that regulating PI3K/AKT signaling pathway alleviated skin inflammation in mice^[12]. One eukaryotic transcription factor that is crucial for controlling inflammatory processes and immunological responses is nuclear factor kappa-B (NF- κ B). Under normal conditions, NF- κ B is isolated in the cytoplasm and attaches itself to the inhibitory proteins inhibitor of NF- κ B (I κ B). Various stimulation can lead to ubiquitin-mediated degradation of I κ B- α to promote NF- κ B activation, ultimately inducing the expression of a series of immune and inflammatory genes^[13]. In this study, dinitrochlorobenzene (DNCB)-induced AD mouse model and lipopolysaccharides (LPS)-induced macrophage inflammation model were involved to investigate the regulation effect and mechanism of IRE on skin inflammation.

2. Materials and methods

2.1 Reagents

Acetone was purchased from Nanjing Chemical Reagent Co. (Nanjing, China). Olive oil was obtained from Shanghai Maclean Biochemical Co. (Shanghai, China). DNCB was purchased from TCI Shanghai Co. (Shanghai, China). Dulbecco's modified eagle medium (DMEM) containing high-glucose, RPMI-1640 medium, minimum essential medium (MEM), fetal bovine serum (FBS), penicillin and streptomycin were purchased from Gibco (USA). TNF- α enzyme-linked immunosorbent assays (ELISA) Kit and

IL-6 ELISA Kit were provided by Multiscience (LIANKE) Biotech, Co. (Hangzhou, China). Anti- β -actin antibody, anti-p-PI3K antibody, anti-PI3K antibody, anti-p-NF- κ B p65 antibody, anti-NF- κ B p65 antibody, anti-p-Akt antibody, anti-Akt antibody, anti-p-I κ B antibody and anti-I κ B antibody were purchased from Cell Signaling Technology (Boston, USA). Anti-rabbit IgG antibody was from Biodragon Immunotech Co. (Nanjing, China). Anti-mouse IgG antibody was purchased from Abcam (Cambridge, UK). Tryptic Soy Agar Medium (TSA) and Tryptic Soy Broth were purchased from Hopebio (Qingdao, China).

2.2 Preparation of extracts

After drying and grinding *Imperatae Rhizoma*, the powder was extracted using ethanol at 80 °C for 2 h. After centrifugation and filtration, the filtrate was concentrated at 45 °C. The extracts were dried with a lyophilizer and stored at -20 °C^[14-15].

2.3 Animals

We purchased female Institute of Cancer Research (ICR) mice aged 6–8 weeks from Nanjing Qinglong Mountain Animal Breeding Farm and kept them in a temperature-controlled environment ((22 $\pm$ 2) °C, 12 h light/dark cycle) with free access to food and water. All animals feeding and the experimental procedures were performed according to the guidelines established by the Animal Ethics Committee of China Pharmaceutical University (2022-11-003).

2.4 Establishment of animal models of AD

Animal models were constructed according to the method of Fan et al.^[16].

According to the workflow in Fig. 1, on the day before the experiment, remove the hair from the back of the mice. On days 1, 2, 3, DNCB was dissolved in acetone:olive oil 3:1 (V/V) base, 100 μ L 1% DNCB was applied to the dorsal skin, and 20 μ L was applied to the outer side of the right ear, while the blank group was given the solvent only. On days 7–21, 100 μ L 0.5% DNCB was applied to the skin of the back and 20 μ L to the outer side of the right ear. The blank group was given the solvent. The dosing group was given 100 μ L 30 mg/mL IRE daily to the dorsal skin and 20 μ L to the lateral side of the right ear, and the blank and model groups were given solvent only. Mice were euthanized on the last day of the experiment.

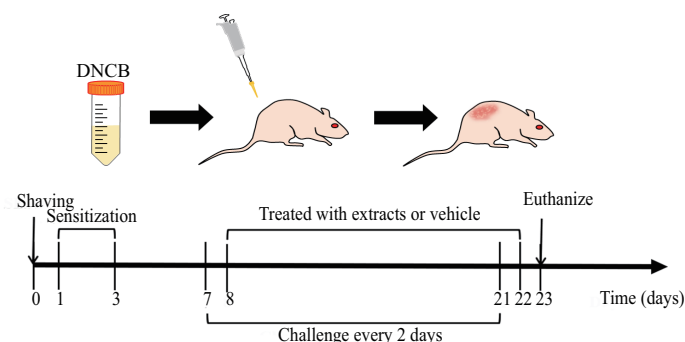


Fig. 1 Roadmap of AD dermatitis experiment in mice.

2.5 Histological analysis

After euthanizing the mice, fix the dorsal skin of the mice with 4% paraformaldehyde and prepare paraffin embedded skin sections. Hematoxylin and eosin (H&E) staining was utilized to assess the epidermal thickness, while toluidine blue staining was employed to determine the extent of mast cell infiltration^[17].

2.6 Liquid mass spectrometry

Liquid chromatography processes: An ACQUITY UPLC BEH C₁₈ 1.7 column (2.1 mm × 100 mm, 1.7 μm) was used with solvent A as H₂O-1% formic acid and solvent B as acetonitrile, and the elution program was 0–37 min, phase A 1%; 37–40 min, phase A 95%.

Mass spectrometry process: the Waters XEVO-G2XS Q-TOF mass spectrometer was employed with the following electrospray ionization (ESI) settings: a mass range of *m/z* 50–1 200, a capillary voltage of 2.5 kV, a sampling cone of 40.000 0, a source temperature of 120 °C, a desolvation temperature of 450 °C, a cone gas flow rate of 50.0 L/h, a desolvation gas flow rate of 800.0 L/h, low mass resolution at 4.7, high mass resolution at 15.0, and a data acquisition time of 40.0 min.

2.7 Network pharmacology analysis

2.7.1 Target of action prediction

The chemical ingredients of the alcoholic extracts of Imperatae Rhizoma were retrieved from the PubChem database. The potential targets of these active ingredients were predicted using the Swiss Target Prediction database. The two-dimensional structure of each compound in Canonical SMILES format was used for the prediction, with the species set as *Homo sapiens*. Targets with a probability value of 0 were excluded from further analysis.

2.7.2 Skin inflammation target collection

Targets related to skin inflammation were collected from the GeneCards and OMIM databases using the keyword “Dermatitis”. The obtained targets were then intersected with the previously identified targets of the active ingredients using a Venn diagram. The intersecting targets were considered as potential therapeutic targets for skin inflammation.

2.7.3 Construction of ingredient-target-disease relationship network diagrams

A visual “ingredient-target-disease” network was constructed using Cytoscape 3.9.1 software. This network integrated the active components of Imperatae Rhizoma with their potential targets associated with skin inflammation, providing a comprehensive overview of the potential therapeutic interactions.

2.7.4 Protein-protein interaction (PPI) network construction and key target screening

The intersecting targets were imported into the STRING database to construct a PPI network. The analysis was performed with the

species set to *Homo sapiens* and the confidence score threshold set to 0.4. The resulting PPI network was then imported into Cytoscape software, and core-acting targets were identified and screened using the CytoNCA tool. This step helped to identify the most significant targets within the network, which are likely to play key roles in the therapeutic effects of the active ingredients^[18].

2.7.5 Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis

To understand the biological processes and signaling pathways involved in the therapeutic effects of the active components, GO function and KEGG pathway enrichment analyses were performed. The intersecting targets were imported into the DAVID database, and the main signaling pathways and biological processes were examined. This analysis provided insights into the potential mechanisms of action of the active components in modulating skin inflammation.

2.8 Cell experiments

2.8.1 Cell culture

RAW264.7 cells were provided by the National Center for Research in Chinese Herbal Medicine, China Pharmaceutical University, and cultured in RPMI-1640 medium containing 10% FBS and 1% penicillin-streptomycin. Human immortalized keratinocyte cell line HaCaT cells, procured from the Cell Resource Center, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences, were cultured in MEM medium containing 10% FBS and 1% penicillin-streptomycin. Cells were cultivated in a biochemical incubator at 37 °C with 5% CO₂.

2.8.2 Cellular activity

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric method was used to investigate the effect of IRE on the activity of RAW264.7 cells and HaCaT cells^[19]. Filled a 96-well plate with 5 000 cells/well. After cells were treated with different doses of IRE for 24 h. Then, according to the instructions in the reagent kit, measure the absorbance at 490 nm.

2.8.3 Determination of NO and ELISA

RAW264.7 cells were inoculated in 96-well plates with 5×10^4 cells per well. The cells were incubated for 24 h, then treated with IRE and 100 ng/mL LPS for 24 h, the culture medium was collected and centrifuged $300 \times g$ at 4 °C for 10 min. The supernatant was added with mixed Gris Reagent A (1% sulfonamide, 2% phosphoric acid) and GrIRE's reagent B (0.1% *N*-(1-naphthyl) ethylenediamine hydrochloride). The absorbance was measured at 540 nm after incubation at room temperature for 30 min. The formula (1) was used to get the inhibition rate:

$$\text{Inhibition rate (\%)} = \frac{\text{OD}_{\text{LPS}} - \text{OD}_{\text{drug}}}{\text{OD}_{\text{LPS}} - \text{OD}_{\text{blank}}} \times 100 \quad (1)$$

Concentrations of IL-6 and TNF- α in the supernatant were determined using ELISA kits, following the protocol provided by the manufacturers.

2.8.4 Western blotting (WB)

RAW264.7 cells were treated with IRE and 100 ng/mL LPS for 24 h, and the protein in cells were extracted with cell lysis buffer containing 1 mmol/L phenylmethylsulfonyl fluoride, protease and phosphatase inhibitors. The concentration of the protein solution was measured with a BCA Protein Assay Kit (Beyotime, P0009), and the protein concentration of the sample solution was calculated based on the curve of the concentration and absorbance of the standard. The grayscale values of protein bands were analyzed using ImageJ.

2.8.5 Cell scratch assay

The HaCaT cells of logarithmic growth phase 5×10^5 cells/well were inoculated in 6-well plates, and the cells were scratched after complete fusion. Serum-free medium containing different concentrations of IRE was added to the well plates, observed under a microscope for 24 h and photographed, and the area of the scratch before and after administration was measured by ImageJ. The formula (2) was used to get the cell migration rate:

$$\text{Cell migration rate (\%)} = \frac{\text{Original area} - \text{Area after scratch}}{\text{Original area}} \times 100 \quad (2)$$

2.9 Bacterial experiments

2.9.1 Staphylococcus aureus revival

S. aureus strain was streaked on TSA agar plates and incubated at 37 °C for 12–24 h to activate and obtain individual colonies. Then selected a single colony and cultured it in Tryptic Soy Broth at 37 °C and 200 r/min until the appropriate turbidity was reached to prepare a bacterial suspension for use.

2.9.2 Determination of growth curve of *S. aureus*

Added the prepared bacterial suspension to 100 mL Tryptic Soy Broth at a ratio of 1:100, and cultured continuously for 12 h at 37 °C and 200 r/min. Took samples every 2 h to measure the OD_{600 nm} value, with blank medium as the control. Plot the growth curve of *S. aureus* with cultivation time as the horizontal axis and OD_{600 nm} as the vertical axis.

2.9.3 Relationship between *S. aureus* colony count and OD_{600 nm}

Added the prepared bacterial suspension to 100 mL Tryptic Soy Broth at a ratio of 1:100, and cultured at 37 °C and 200 r/min. Took logarithmic growth stage bacteria according to the growth curve, serial diluted 6 times, and measured their OD_{600 nm} respectively. And took 50 μ L the dilution solution of 1×10^5 and 1×10^6 CFU/mL to TSA agar plates respectively. After culturing for 12–24 h, counted the colonies of *S. aureus*^[20]. Plotted the relationship curve between *S. aureus* OD_{600 nm} and number of colonies using OD_{600 nm} as the horizontal axis and number of colonies as the vertical axis.

2.9.4 Determination of antibacterial activity of *S. aureus*

Using the double dilution method to determine the antibacterial activity of IRE on *S. aureus*. Took a sterile 96-well plates, added 100 μ L of sample solution to well 1, added 100 μ L of Tryptic Soy Broth to wells 2 to 10, added another 100 μ L of sample solution to well 2, mix well, and then draw 100 μ L to well 3 for sequential dilution to well 10. Each gradient was repeated 3 times, and 1 μ L of 1×10^6 – 1×10^7 CFU/mL bacterial suspension was added to wells 1 to 11. Among them, the well without bacterial suspension is the positive control, and the well without IRE is the negative control. After vaccination, incubated at 37 °C for 12 h. Took out 50 μ L of bacterial solution from each well and diluted it in a gradient to 1×10^6 – 1×10^7 CFU/mL. Took 50 μ L of the dilution solution to TSA agar plates. After culturing for 12–24 h, counted the colonies of *S. aureus*.

2.10 Statistical analysis

The statistical analysis for this study was conducted using Prism 8 (GraphPad Software Inc., USA). A One-way analysis of variance (ANOVA) coupled with Duncan's multiple range test was utilized to analyze the data. The results were presented as the mean $\pm$ standard deviation (SD) based on three replicates. To denote the significance of the findings, * indicated $P < 0.05$, ** indicated $P < 0.01$, and *** indicated $P < 0.001$ ^[21].

3. Results

3.1 IRE significantly reduces DNCB-induced skin inflammation in ICR mice

As shown in Fig. 2A, mice in the model group had massive crusting and flaking, and the skin showed a dry, rough and red state. The scabbing area of the IRE administration group was significantly smaller, and the dry and red state of the skin had a certain recovery effect (Fig. 2A). The inflammation scores of the model mice increased steadily, while the inflammation scores of the IRE administration group significantly decreased and showed a significant trend (DNCB: 4.80 ± 1.09 , IRE: 3.00 ± 1.26), indicating that IRE improved the skin inflammation condition of the mice and reduced the manifestation of swelling erythema dry flaking (Fig. 2B). The thickness of the mice ears reflected the change in the degree of swelling^[22]. Relative to the blank group, the degree of ear swelling was higher in the model group mice and lower in the IRE administration group with a significant trend (Fig. 2C). The scratching behavior of mice at the modeled site is one of the primary symptomatic manifestations in AD model mice^[23–24]. The number of scratching was visibly higher in the model group, and the IRE administration group showed an improvement trend in pruritus (Fig. 2D).

The spleen is a crucial immunological organ, and the literature indicates that the spleen may be enlarged in AD mice, indicating abnormal immune function^[25]. As shown in Fig. 2E, the spleen index of the model mice increased, but did not decrease significantly after the administration of IRE, although there was a downward trend.

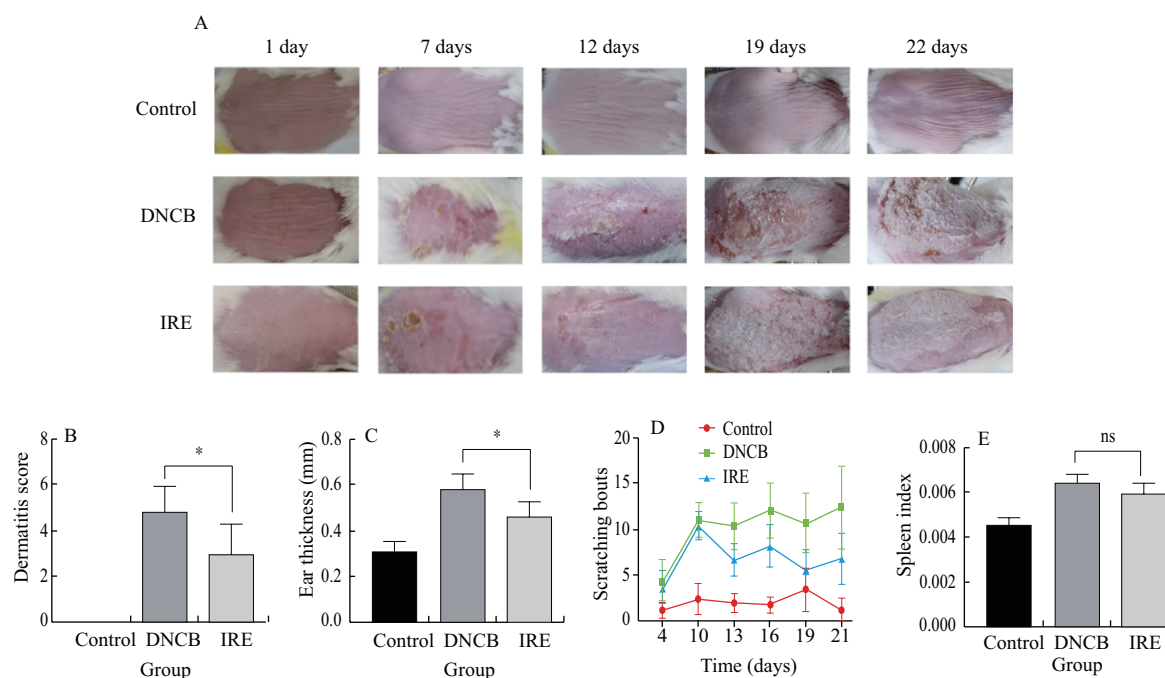


Fig. 2 Ameliorative effect of IRE on mice dermatitis. (A) Apparent effect of IRE on the skin of mice back; (B) Number of scratches in 15 min; (C) Mice ear thickness; (D) Mice skin inflammation score in 15 min; (E) Mice spleen index. ns. not significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

3.2 IRE affects the inflammatory cell infiltration and pathological changes in mice skin

Based on the H&E staining results, the skin structure was intact at all levels and the stratum corneum was not significantly shed in the control group. However, the skin structure was disturbed, the condition of stratum corneum shed was obvious, the layer of spiny cells increased, and the epidermal layer was significantly thickened in the model group (Fig. 3A). The thickened epidermis was significantly improved by IRE administration (control: 17.96 ± 1.797 μm , DNCB: 64.11 ± 1.997 μm , IRE: 47.54 ± 1.539 μm) (Fig. 3B). It has been reported that the number of mast cells in the skin lesions of AD model mice increases significantly and the infiltration degranulation is more severe^[26-27]. In the control group, a few purple-stained mast

cells were seen without significant granule release; In the model group, the number of mast cells was increased and the infiltration of degranulation was obvious significantly (Fig. 3C); In the IRE administration group, the number of mast cells was significantly reduced (Fig. 3D).

3.3 Network pharmacology analysis of targets and mechanisms of IRE

The liquid chromatography-mass spectrometry combined with the self-built database comparison results inferred 14 components (Table 1), of which 14 components identified were mainly phytosterols and triterpenes, and also contained some phenolic components, alkaloids and other components (Fig. 4).

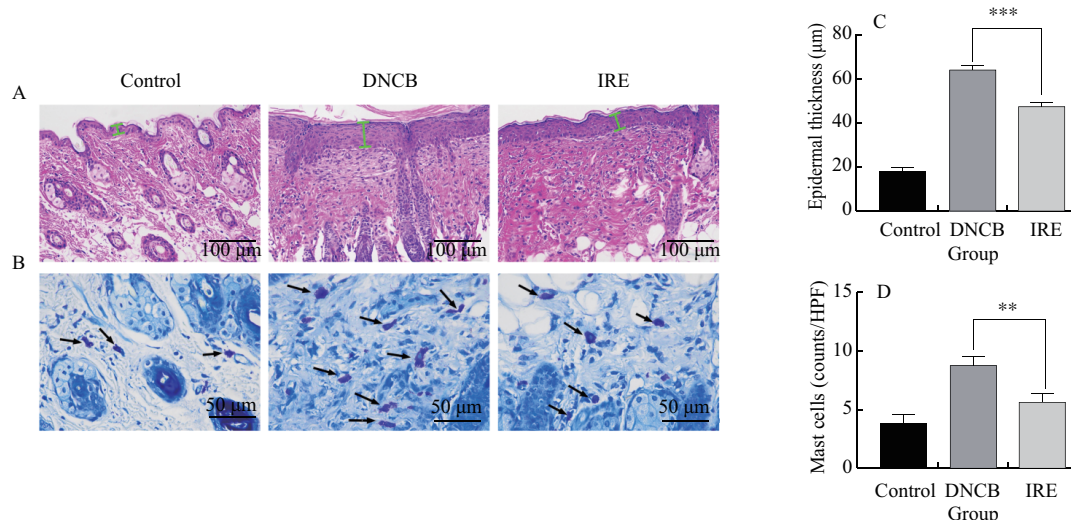


Fig. 3 IRE improved the pathological features of mice skin. (A) H&E-stained section (200 $\times$); (B) Toluidine blue-stained section (400 $\times$); (C) Epidermal layer thickness; (D) Number of mast cells in the field of view.

Table 1
Identification of main components in IRE extract by LC-MS.

Peak	Retention time (min)	Molecular formula	Quasimolecular ion (m/z)		Identification
			Measured	δ	
1	1.81	C ₁₀ H ₈ O ₄	[M-H] ⁻ 251.056 47	1.42	Anemonin
2	2.78	C ₁₂ H ₁₂ O ₄	[M+H] ⁺ 223.096 32	-0.76	Siderin
3	2.96	C ₁₆ H ₁₈ O ₉	[M+H] ⁺ 355.102 17	-0.53	Chlorogenic acid
4	3.37	C ₁₅ H ₁₁ ClO ₅	[M-H] ⁻ 365.045 98	7.18	Luteolindin
5	4.53	C ₉ H ₈ O ₃	[M-H] ⁻ 223.060 98	-0.99	Anti-coumaric acid
6	5.31	C ₈ H ₇ NO ₃	[M+H] ⁺ 183.076 71	1.57	Coixol
7	6.16	C ₁₉ H ₂₂ O ₅	[M-H] ⁻ 329.139 67	0.67	Imperanene
8	20.97	C ₁₅ H ₂₀ O ₂	[M-H] ⁻ 277.144 85	1.16	Cylindrene
9	25.67	C ₂₈ H ₄₈ O	[M+H] ⁺ 423.359 97	0.56	Campesterol
10	28.99	C ₃₀ H ₄₈ O	[M+H] ⁺ 425.378 20	0.97	Arborinone
11	31.80	C ₃₀ H ₅₀ O	[M+H] ⁺ 427.393 36	-0.20	Isoarborinol
12	32.48	C ₂₉ H ₄₈ O	[M-H] ⁻ 457.368 91	0.42	Stigmasterol
13	32.68	C ₂₉ H ₅₀ O	[M+H] ⁺ 397.381 82	-2.67	β -Sitosterol
14	33.91	C ₃₀ H ₅₀ O	[M+H] ⁺ 427.393 86	0.97	Simiarenol

Note: δ refers to the mass accuracy (mass deviation), which measures the degree of agreement between the experimentally measured molecular weight and the the oretically calculated molecular weight. In chemical identification, $|\delta| \leq 5$ is generally regarded as high-accuracy confirmation.

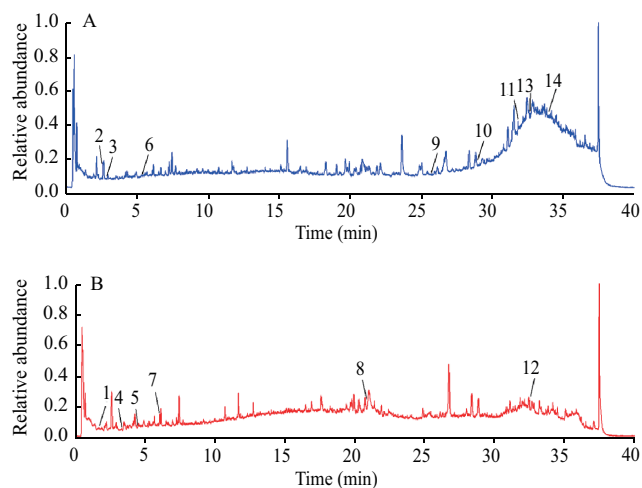


Fig. 4 Total ion chromatogram of IRE (A) positive and (B) negative modes.

By searching the Swiss Target Prediction database for the 14 components found in IRE, 198 potential targets were found. By de-duplicating the GeneCards database with the skin inflammation-related targets that were retrieved from the OMIM database, 3 083 targets were found, and a Venn diagram showed 195 intersecting targets (Fig. 5A). Fig. 5B displays the results of constructing a network with PPI that were entered into the String database. CytoNCA tool was applied to analyze and screen the core-acting targets, and the top 20 targets with high scores are shown in Fig. 5C, among which the key targets with high scores are TNF, AKT1, sarcoma kinase (SRC), epidermal growth factor receptor (EGFR), etc. The common targets were imported into the DAVID database and then ranked according to the magnitude of their $-\lg P$ values. The top ranked biological processes as shown in the Fig. 5D include response to drugs, inflammatory response, peptidyl tyrosine phosphorylation, response to lipopolysaccharide, response to exogenous stimuli, and the top ranked molecular functions as shown in the Fig. 5E. As shown in the Fig. 5F, the top ranked cellular components include components of the plasma membrane, membrane rafts, cytoplasmic lysates, macromolecular complexes, and plasma membrane.

A total of 165 signaling pathways were enriched and ranked according to the magnitude of their $-\lg P$ values, as shown in the Fig. 5G, the top 10 ranked signaling pathways include cancer pathways, lipids and atherosclerosis, Kaposi's sarcoma-associated herpesvirus infection, Th17 cell differentiation, advanced glycation end products-receptor for advanced glycation end products (AGE-RAGE) signaling pathway in diabetic complications, programmed cell death ligand 1 (PD-L1) expression in cancer and programmed cell death protein 1 (PD-1) checkpoint pathway, endocrine resistance, tumor necrosis factor signaling pathway, hepatitis B, and T-cell receptor signaling pathway.

3.4 IRE suppressed the production of inflammatory factors *in vitro*

In the constructed LPS-stimulated RAW264.7 cells, the MTT assay was used to assess the cytotoxicity of IRE. As shown in the Fig. 6A, IRE had no obvious cytotoxicity and showed a certain promoting effect on cell proliferation, as evidenced by 100, 200 and 400 $\mu\text{g/mL}$ promoted cell activity and showed some dose dependence (50 $\mu\text{g/mL}$: $(97.730 \pm 7.005)\%$, 100 $\mu\text{g/mL}$: $(113.500 \pm 2.958)\%$, 200 $\mu\text{g/mL}$: $(118.700 \pm 5.041)\%$, 400 $\mu\text{g/mL}$: $(131.300 \pm 2.076)\%$).

NO was measured using the GriRE's reagent method^[28], and the ration of NO production decreased to $(90.80 \pm 4.61)\%$, $(83.20 \pm 2.22)\%$, and $(75.47 \pm 3.00)\%$ respectively when IRE concentration was 50–200 $\mu\text{g/mL}$ (Fig. 6B). The levels of TNF- α and IL-6, 2 important inflammatory factors, indicate the inflammatory response intensity^[29-30]. As shown in Figs. 6C and D, LPS induction significantly increased the release of TNF- α and IL-6, whereas they gradually decreased with the increase of IRE concentration. TNF- α and IL-6 expression decreased from (264.00 ± 10.77) and (45.160 ± 2.795) pg/mL in the LPS group to (162.900 ± 4.893) and (14.150 ± 0.199) pg/mL (IRE 200 $\mu\text{g/mL}$), respectively. The inducible nitric oxide synthase (iNOS) expression was measured and showed a dose-dependent decrease with increasing IRE concentration. iNOS expression reached $(73.670 \pm 5.242)\%$, $(60.390 \pm 5.985)\%$, $(44.33 \pm 10.44)\%$ of the model group when IRE concentration was at 50, 100, 200 $\mu\text{g/mL}$ (Figs. 6E and F).

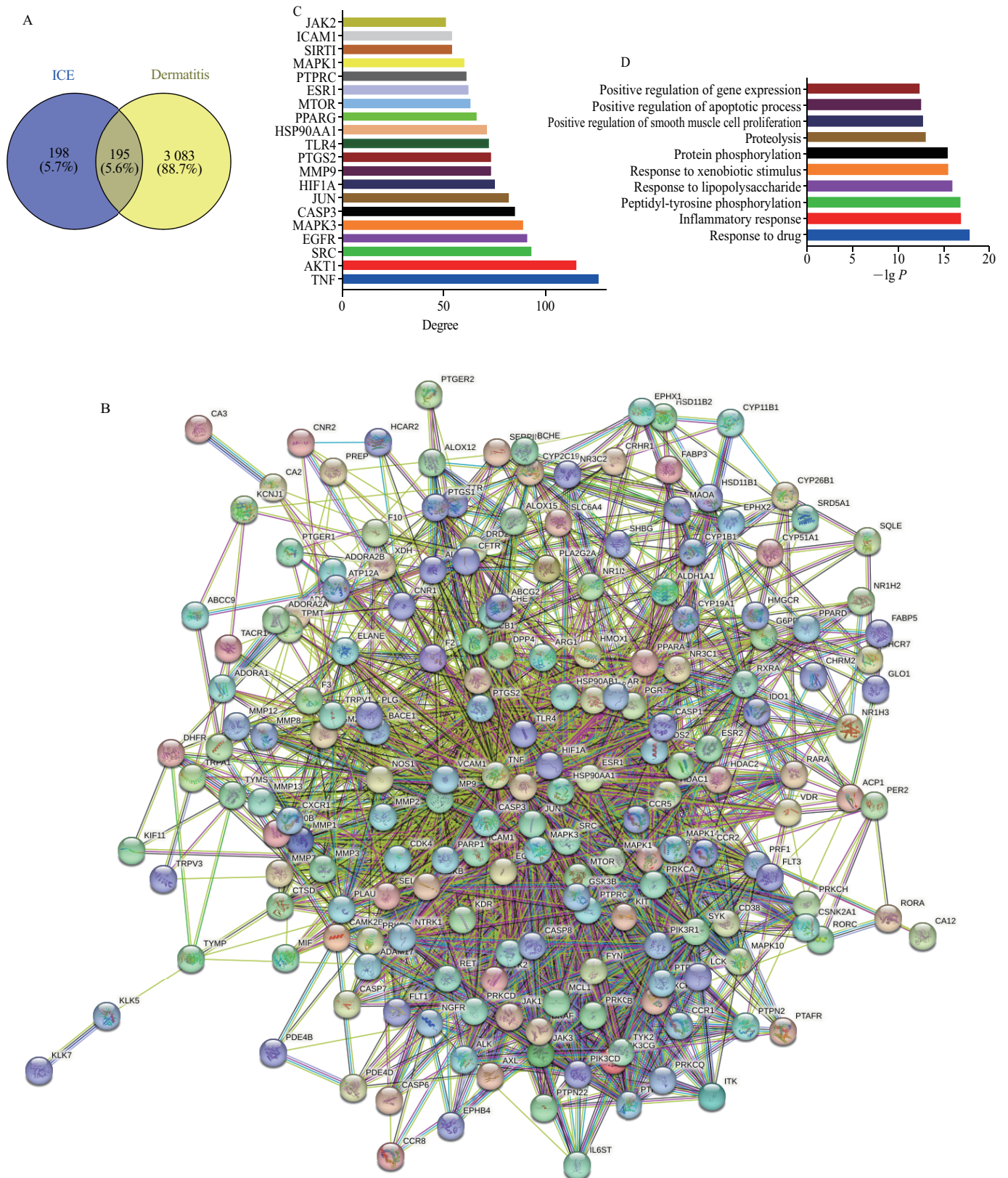


Fig. 5 Network pharmacology analysis of IRE component-target network. (A) Venn diagram of IRE components and skin inflammation targets; (B) STRING network of PPI; (C) Component-target top 20 intersection targets; (D) GO biological process; (E) GO molecular function; (F) GO cellular component; (G) KEGG pathway enrichment results.

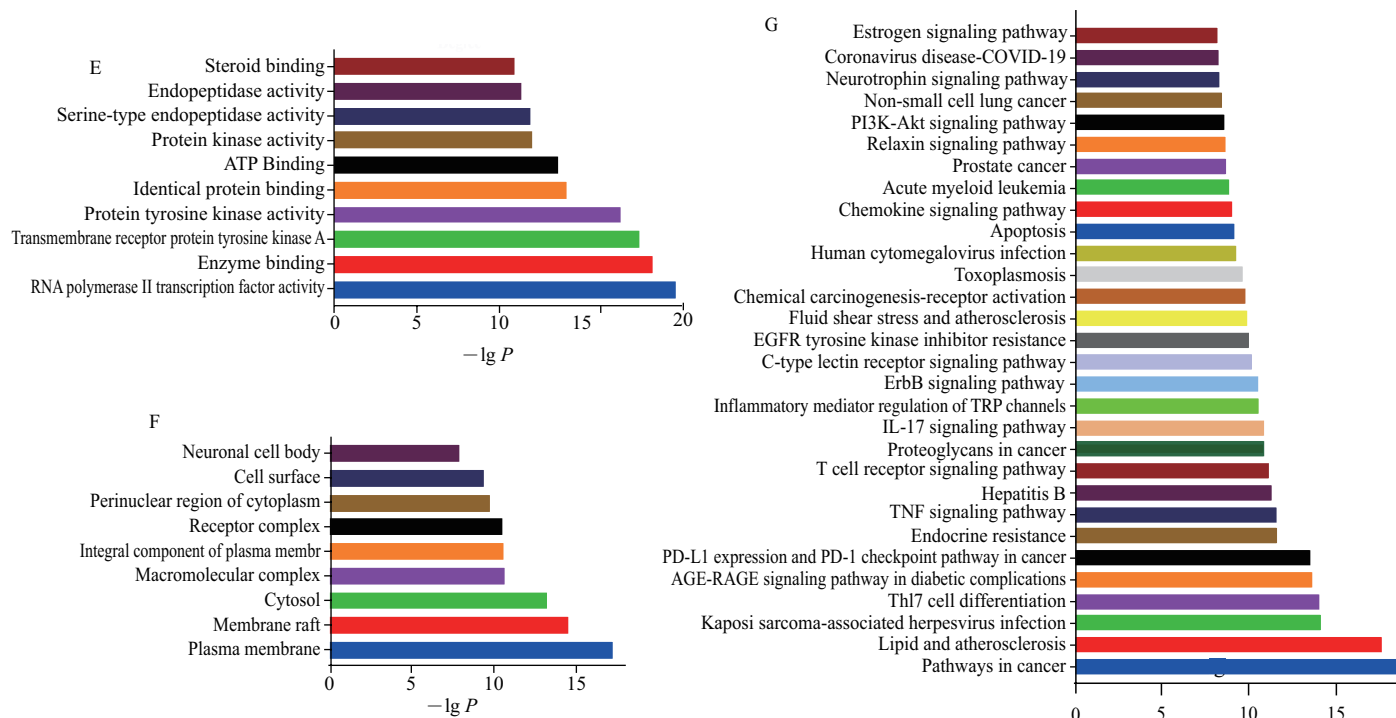


Fig. 5 (Continued)

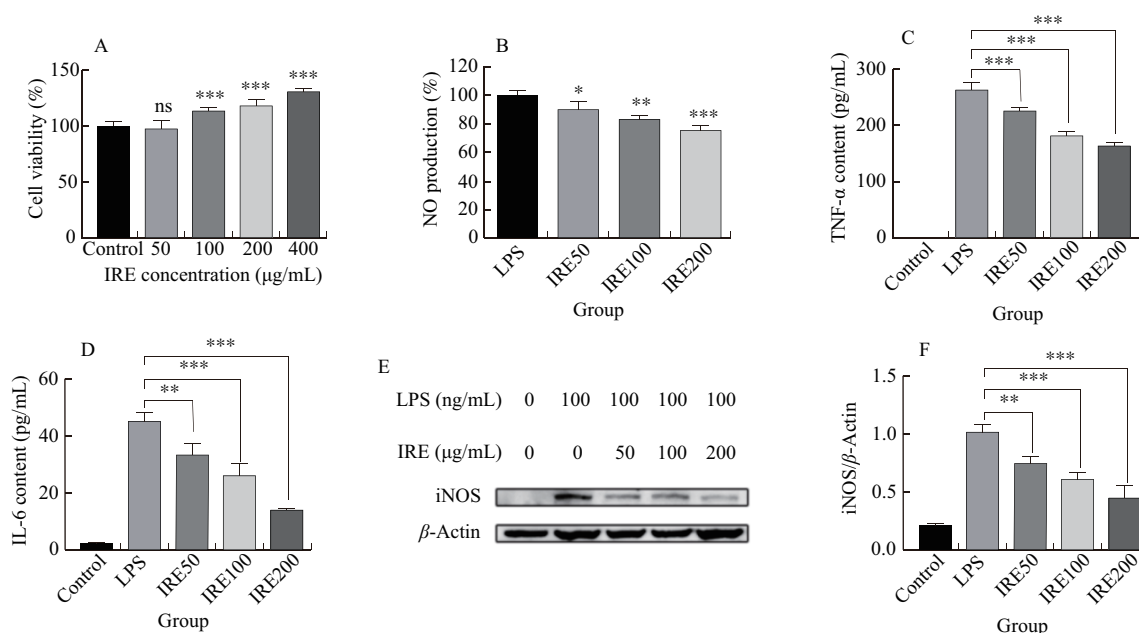


Fig. 6 Anti-inflammatory activity of IRE *in vitro*. (A) Effect of IRE on RAW264.7 cell activity; (B) Effect of IRE on LPS-induced NO production in RAW264.7 cells; (C, D) Effect of IRE on LPS-induced release of inflammatory factors TNF-α and IL-6 in RAW264.7 cells; (E, F) Effect of IRE on LPS-induced iNOS protein expression in RAW264.7 cells.

3.5 IRE inhibits inflammatory responses via PI3K/AKT/NF-κB

The signaling pathways were confirmed in conjunction with the findings of the network pharmacology analysis. The relevant phosphorylation and total protein expression were determined by western blotting, and the experimental results were shown in Fig. 7A. Treatment with varying concentrations of IRE could decrease the proportion of phosphorylated PI3K and phosphorylated AKT, and the

inhibition effect demonstrated a dose-dependent effect. The model group's phosphorylation of PI3K and AKT was higher than that of the control group significantly (Figs. 7B and C). The effect of IRE on NF-κB pathway was researched and IRE treatment of RAW264.7 cells could reduce the proportion of phosphorylated NF-κB as well as phosphorylated IκB, and the inhibition effect showed a dose dependence (Figs. 7D and E).

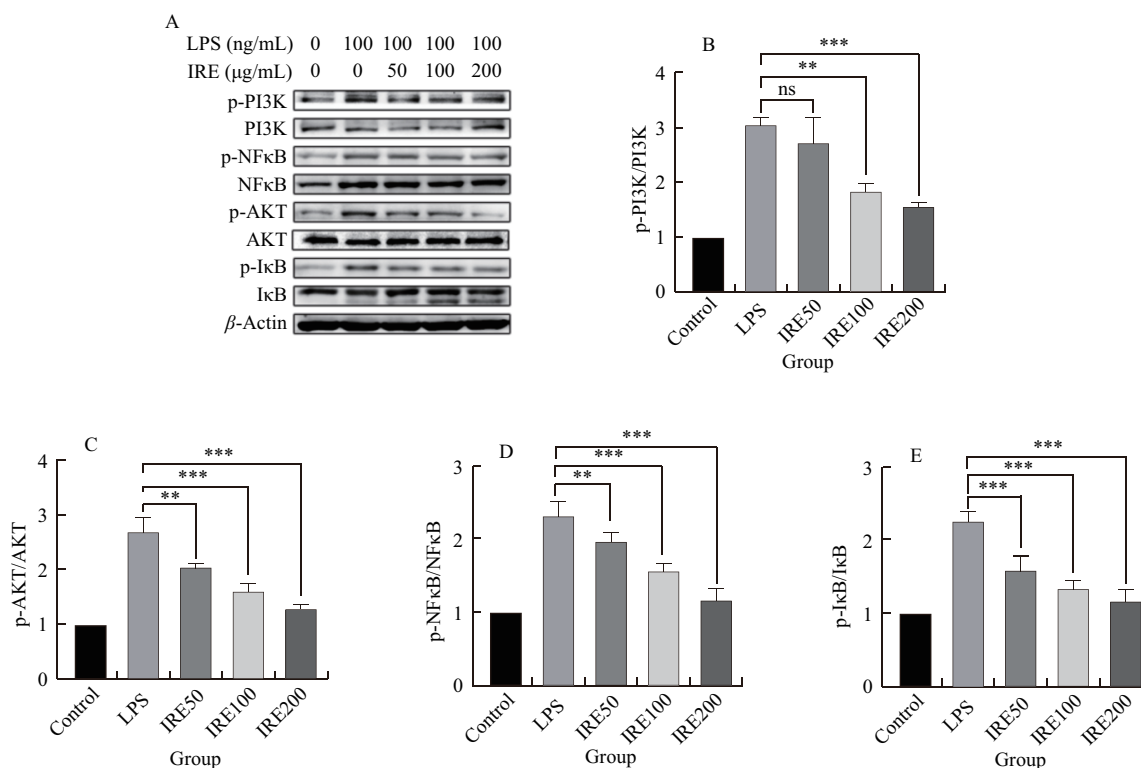


Fig. 7 IRE inhibited PI3K/AKT/NF-κB signaling pathway in LPS-induced RAW264.7 cells. (A) The expression of p-PI3K/PI3K, p-AKT/AKT, p-NFκB/NFκB, p-IκB/IκB in LPS-induced RAW264.7 cells. (B-E) Quantized histogram of p-PI3K/PI3K, p-AKT/AKT, p-NFκB/NFκB, p-IκB/IκB.

3.6 IRE promotes cell migration capacity

It has been reported in the literature that the increased ability of HaCaT cells may contribute to the healing of skin wounds^[31-32]. Firstly, in order to exclude the interference of cytotoxicity to the experiment, the HaCaT cell activity was measured with different concentrations of IRE. The experimental results were shown in the Fig. 8A, the effect of IRE concentration below 300 μg/mL was not significant on the activity of HaCaT cells, from 400 μg/mL onwards showed significant cytostatic, and showed a certain dose dependence (control: $(100.000 \pm 6.438)\%$, 100 μg/mL: $(97.820 \pm 4.615)\%$, 200 μg/mL: $(92.470 \pm 6.463)\%$, 300 μg/mL: $(88.280 \pm 7.541)\%$, 400 μg/mL: $(80.800 \pm 6.574)\%$ and 500 μg/mL: $(75.05 \pm 11.05)\%$). The effect of IRE on the migration of HaCaT cells was evaluated by scratch assay. When the IRE concentration was 25 μg/mL, the cell migration ability of HaCaT was not changed significantly, but when the IRE concentration was 50 and 100 μg/mL, the cell migration ability of HaCaT was significantly increased (Fig. 8B). This indicated that a certain concentration of IRE could promote wound healing of HaCaT cells (control: $(34.510 \pm 4.088)\%$, 25 μg/mL: $(39.150 \pm 6.205)\%$, 50 μg/mL: $(42.350 \pm 5.591)\%$, 100 μg/mL: $(43.160 \pm 4.645)\%$) (Fig. 8C).

3.7 Antibacterial activity of IRE

According to the method in Section 2.9.2, the growth status of *S. aureus* at different times was measured. The growth curve of

S. aureus was plotted with time as the x-axis (x) and the absorbance value of OD_{600 nm} as the y-axis (y). From Fig. 9A, it could be seen that the logarithmic growth period of *S. aureus* is 3–4 h. Fig. 9B showed a linear relationship between the number of *S. aureus* colonies and OD_{600 nm}, with a regression equation of $y = 6.4649x - 0.4953$, $R^2 = 0.9929$. The logarithmic growth period bacteria could be diluted to the appropriate bacterial solution concentration according to the regression equation. When the concentration of *S. aureus* is $1 \times 10^6 - 1 \times 10^7$ CFU/mL, the OD_{600 nm} is 0.077–0.092.

From Figs. 9C and D, it could be seen that IRE exhibits proliferative activity against *S. aureus* at concentrations ranging from 0.390 615–12.5 mg/mL, and showed a trend of increasing and then decreasing with increased concentration. IRE inhibited the growth of *S. aureus* with a concentration of 25 mg/mL. However, at concentrations ranging from 50 to 200 mg/mL, there was no bacterial growth observed on TSA agar plates, indicating that IRE had a significant antibacterial effect on *S. aureus*. IRE contains various active substances such as polysaccharides, plant sterols, triterpenes, polyphenols, alkaloids, etc., which were the main components that exert antibacterial effects in IRE^[33]. At low concentrations, the polysaccharides with higher content in IRE may provide nutrients for bacteria, while the antibacterial active ingredients have relatively lower content and cannot exert antibacterial effects. At high concentrations, IRE contains a relatively high amount of antibacterial active ingredients, with significant antibacterial effects and even bactericidal effects.

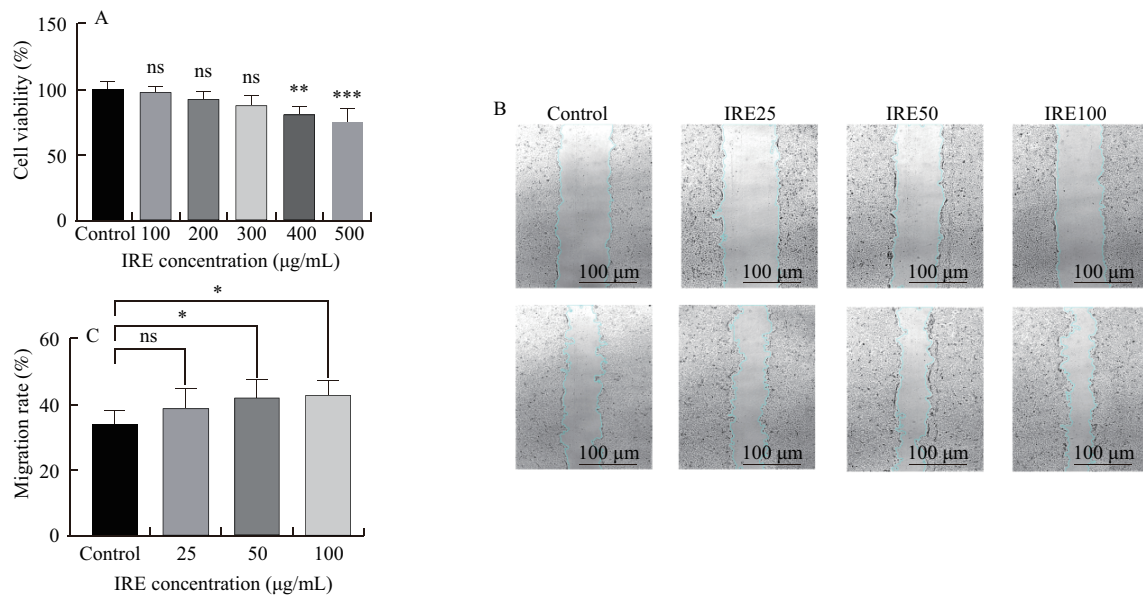


Fig. 8 IRE enhanced the cell migration capacity of HaCaT cells. (A) Effect of IRE on the activity of HaCaT cells; (B) Image of the migration ability of IRE on HaCaT cells (40×); (C) Effect of IRE on the migration rate of HaCaT cells.

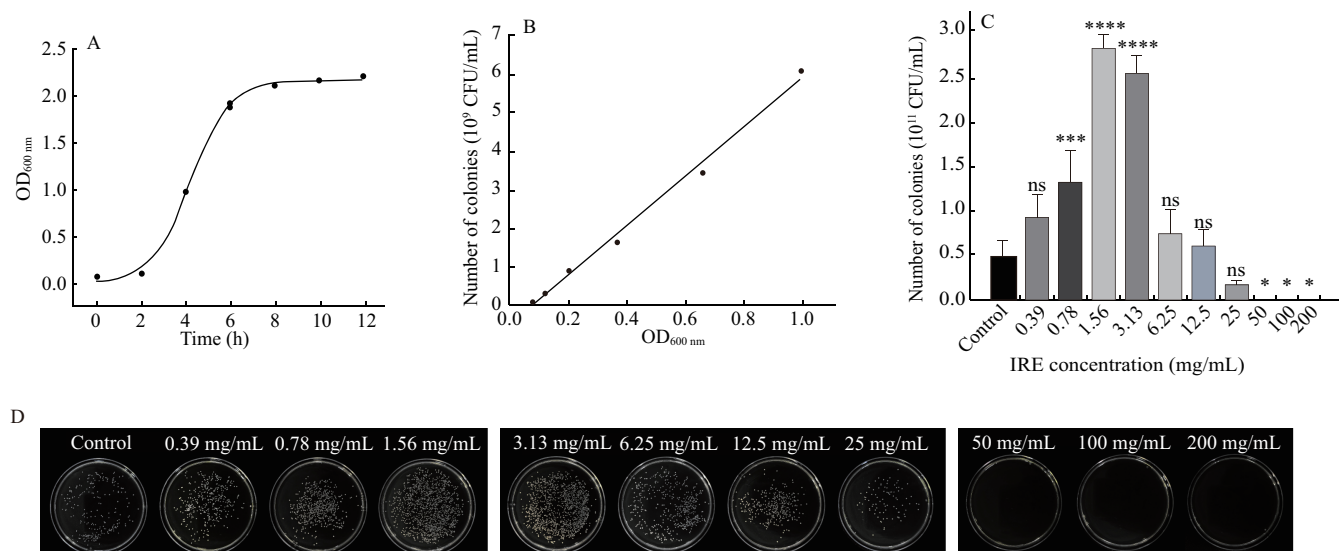


Fig. 9 Inhibitory activity of IRE on *S. aureus*. (A) Growth curve of *S. aureus*; (B) Relationship between *S. aureus* OD_{600 nm} and number of colonies; (C) Effect of IRE with different concentrations on the number of colonies of *S. aureus* compared with control; (D) TSA agar plates of different concentrations of IRE to *S. aureus*.

4. Discussion

4.1 Core targets and their biological pathway analysis in AD Treatment

PPI topological network analysis revealed that the core targets of IRE bioactive compounds for the AD treatment include TNF, AKT1, SRC, EGFR, MAPK3, etc. AKT1 knockdown significantly increases pro-inflammatory cytokines TNF- α , IL-6 and IL-1 β production, which exacerbates renal tubular apoptosis and inflammatory response^[34]. EGFR is a membrane tyrosine kinase receptor, and inhibition of EGFR has been documented to inactivate NF- κ B, thereby inhibiting iNOS expression and the production of pro-inflammatory cytokines,

which exerting anti-inflammatory activity^[35-36]. MAPK3 is a mitogen activated protein kinase. It has been reported that inhibition of MAPK3 alleviates intestinal inflammation^[37-38].

GO functional analysis revealed that these targets are mainly associated with inflammatory responses, responses to lipopolysaccharides, responses to exogenous stimuli, protein phosphorylation, nitric oxide biosynthesis, and positive regulation of cell migration. According to KEGG data, the active ingredients in IRE may inhibit dermatitis *via* a variety of pathways, including AGE-RAGE, TNF, T-cell receptors, IL-17, TRP, PI3K-AKT, chemokines, NF- κ B, and the estrogen signaling pathway.

It was reported that PI3K/AKT and mammalian target of rapamycin (mTOR) kinases were activated, peroxisome proliferator-

activated receptor beta/delta (PPAR β/δ) and fatty acid-binding protein 5 (FABP5) were upregulated in psoriasis-like dermatitis^[39]. According to the literature, PI3K/AKT/mTOR pathway dysregulation may worsen inflammatory symptoms and cause uncontrolled skin proliferation. This has brought some attention to the signaling cascade pathway of PI3K/AKT/mTORC1 in skin inflammatory diseases. TNF- α and other TNF superfamily cytokines would enhance the type 2 immune response^[40]. The type 2 immune response, Th2 differentiation, IgE production, and the activity of innate type 2 effectors are all significantly impacted by the NF- κ B signaling pathway. One of the important mechanisms of AD is the enhanced Th2 response, followed by the secretion of various cytokines, which in turn promotes IgE production and affects the skin barrier-related proteins expression, such as FLG and tight junction proteins^[41–42].

4.2 Identification of primary active ingredients in IRE

Cylindrene, luteolinidin, and 4,7-dimethoxy-5-coumarin in IRE are linked to the most core targets, indicating that they might be the primary active ingredients in the AD therapy^[43]. Luteolinidin is obtained as a salt of the flavonoid lignocaine, which has been shown to exert intestinal anti-inflammatory effects by Janus kinase/signal transducer and activator of transcription (JAK/STAT) pathway inhibition^[44]. 4,7-Dimethoxy-5-coumarin is a coumarin-like component with antibacterial activity as well as antitumor activity^[45]. Cylindrene has been shown to inhibit thrombin-induced platelet aggregation as well as tyrosinase activity in rabbits, but its anti-inflammatory pharmacological activity has not been studied.

The core targets identified through PPI network analysis and the pathways elucidated by KEGG analysis provide a comprehensive understanding of the potential mechanisms of IRE in AD treatment. The primary active ingredients, such as cylindrene, luteolinidin, and 4,7-dimethoxy-5-coumarin, warrant further investigation to elucidate their anti-inflammatory activities. Future studies should focus on validating the specific roles of these active ingredients and their mechanisms of action to fully harness the therapeutic potential of IRE in AD treatment.

5. Conclusion

This study demonstrates that IRE alleviates AD symptoms in a DNCB-induced mouse model by reducing skin dryness, itching, and ear swelling, and modulating epidermal thickening and mast cell infiltration. IRE contains 14 components, mainly phytosterols and triterpenes. Network pharmacology and *in vitro* analysis confirm that IRE's anti-inflammatory effects involve the PI3K/AKT/NF- κ B pathway, inhibiting NO, iNOS, TNF- α , and IL-6. IRE also promotes HaCaT cell migration, accelerates skin healing, and inhibits *S. aureus*. Thus, IRE is a potential AD treatment by reducing inflammation and regulating skin microbiota.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

- [1] J.I. Silverberg, L.S. Gold, S. Desai, et al., Disease burden and patient characteristics associated with systemic therapy utilization among adults with atopic dermatitis: data from CorEvitas Atopic Dermatitis Registry, *J. Dermatol. Treat.* 35 (2024) 96382. <https://doi.org/10.1080/09546634.2024.2396382>.
- [2] S. Kasraie, T. Werfel, Role of macrophages in the pathogenesis of atopic dermatitis, *Mediat. Inflamm.* 2013 (2013) 942375. <https://doi.org/10.1155/2013/942375>.
- [3] Y. Teng, H.T. Zhong, X.H. Yang, et al., Current and emerging therapies for atopic dermatitis in the elderly, *Clin. Interv. Aging* 18 (2023) 1641–1652. <https://doi.org/10.2147/CIA.S426044>.
- [4] P. Kumar, M.S. Ashawat, A. Kumar, et al., Monoclonal antibodies: current advancements and future potential for atopic dermatitis therapy, *Curr. Pharm. Biotechnol.* 26 (2025) 1617–1633. <https://doi.org/10.2174/0113892010311098240530104530>.
- [5] H. Shah, F.J. Eckembrecher, D.G. Eckembrecher, et al., Current and emerging immunobiologic therapies for atopic dermatitis, *Drugs Ther. Perspect* 40 (2024) 1–12. <https://doi.org/10.1007/s40267-024-01075-8>.
- [6] Z.X. Cao, Y.X. Li, A.J. Ma, et al., Analysis and comparison of staminate flowers components in five Chinese walnut varieties, *Food Med. Homol.* 1 (2024) 9420005. <https://doi.org/10.26599/FMH.2024.9420005>.
- [7] H.Y. Jiang, H.N. Cao, J.Y. Ruan, et al., Isolation and characterization of anti-inflammatory chromones from *Imperata Cylindrica* var. major, *Chem. Nat. Compd.* 58 (2022) 623–627. <https://doi.org/10.1007/s10600-022-03756-0>.
- [8] W.C. Yu, J.F. Li, Y. Xiong, et al., Optimization of ultrasound-assisted extraction of *Imperata Cylindrica* polysaccharides and evaluation of its anti-oxidant and amelioration of uric acid stimulated cell apoptosis, *Ultrason. Sonochem.* 104 (2024) 106844. <https://doi.org/10.1016/j.ulsonch.2024.106844>.
- [9] J.W. Zhou, K.Y. Yin, W.Q. Luo, et al., Anti-virulence potential of carvone against *Serratia marcescens*, *Food Med. Homol.* 1 (2024) 9420001. <https://doi.org/10.26599/FMH.2024.9420001>.
- [10] C.N. Zumbi, C.H. Pan, H.Y. Huang, et al., *Ascophyllum nodosum* and *Fucus vesiculosus* ameliorate restenosis via improving inflammation and regulating the PTEN/PI3K/AKT signaling pathway, *Food Sci. Hum. Wellness* 13 (2024) 1711–1728. <https://doi.org/10.26599/FSHW.2022.9250222>.
- [11] Y.Y. Zhao, J.F. Wei, C.Q. Li, et al., A comprehensive review on mechanism of natural products against *Staphylococcus aureus*, *J. Future Foods* 2 (2022) 33–41. <https://doi.org/10.1016/j.jfutfo.2022.03.014>.
- [12] Y.W. Lin, X.F. Zhu, Y.W. Li, et al., LY294002 ameliorates psoriatic skin inflammation in mice via blocking the Notch1/Hes1-PTEN/AKT/IL-17A feedback loop, *Clin. Exp. Immunol.* 213 (2023) 114–124. <https://doi.org/10.1093/cei/uxad025>.
- [13] R.N. Raka, Z.Q. Ding, Y. Yuan, et al., Pingyin rose essential oil alleviates LPS-Induced inflammation in RAW264.7 cells via the NF- κ B pathway: an integrated *in vitro* and network pharmacology analysis, *Bmc. Complement Med.* 22 (2022) 272. <https://doi.org/10.1186/s12906-022-03748-1>.
- [14] P. Nayim, A.T. Mbaveng, M. Sanjukta, et al., *CD24* gene inhibition and *TIMP-4* gene upregulation by *Imperata Cylindrica*'s root extract prevents metastasis of CaSki cells via inhibiting PI3K/Akt/snail signaling pathway and blocking EMT, *J. Ethnopharmacol.* 275 (2021) 114111. <https://doi.org/10.1016/j.jep.2021.114111>.
- [15] Y. Yao, F. Chen, M.F. Wang, et al., Antidiabetic activity of mung bean extracts in diabetic KK-Ay Mice, *J. Agric. Food Chem.* 56 (2008) 8869–8873. <https://doi.org/10.1021/jf8009238>.

- [16] P.L. Fan, Y.L. Yang, T. Liu, et al., Anti-atopic effect of *Viola yedoensis* ethanol extract against 2,4-dinitro-chlorobenzene-induced atopic dermatitis-like skin dysfunction, *J. Ethnopharmacol.* 280 (2021) 114474. <https://doi.org/10.1016/j.jep.2021.114474>.
- [17] X.L. Ji, C.Y. Hou, Y.G. Gao, et al., Metagenomic analysis of gut microbiota modulatory effects of jujube (*Ziziphus jujuba* Mill.) polysaccharides in a colorectal cancer mouse model, *Food Funct.* 11 (2020) 163. <https://doi.org/10.1039/c9fo02171j>.
- [18] L. Wang, Y. Li, W. Wang, et al., Study on the mechanism of Zhibai Dihuang Pills in treatment of endocrine resistance in breast cancer based on network pharmacology, *Drugs and Clinic* 39 (2024) 69-80. <https://doi.org/10.7501/j.issn.1674-5515.2024.01.009>.
- [19] Z.L. Xie, Y. Wang, J.Q. Huang, et al., Anti-inflammatory activity of polysaccharides from *Phellinus linteus* by regulating the NF- κ B translocation in LPS-stimulated RAW264.7 macrophages, *Int. J. Biol. Macromol.* 129 (2019) 61-67. <https://doi.org/10.1016/j.ijbiomac.2019.02.023>.
- [20] Y.Y. Zhao, D.D. Xia, P.P. Ma, et al., Advances in the detection of virulence genes of *Staphylococcus aureus* originate from food, *Food Sci. Hum. Wellness* 9 (2020) 40-44. <https://doi.org/10.1016/j.fshw.2019.12.004>.
- [21] Q.P. Zhang, W.Y. Chen, B.Y. Zhang, et al., Central role of TRAP1 in the ameliorative effect of oleanolic acid on the mitochondrial-mediated and endoplasmic reticulum stress-excited apoptosis induced by ochratoxin A, *Toxicology* 450 (2021) 152681. <https://doi.org/10.1016/j.tox.2021.152681>.
- [22] A.A. Kostiala, delayed-type hypersensitivity measured as an increase of ear thickness in guinea-pigs, *Int. Arch. Aller. Imm.* 60 (1979) 207-215. <https://doi.org/10.1159/000232343>.
- [23] X. Chen, C.Y. Yang, J.Q. Zeng, et al., The protective effects of human milk components, 2'-fucosyllactose and osteopontin, against 2,4-dinitrochlorobenzene-induced atopic dermatitis in mice, *J. Funct. Foods* 87 (2021) 104806. <https://doi.org/10.1016/j.jff.2021.104806>.
- [24] X.L. Zhang, H.L. Ma, J. Quaisie, et al., Tea saponin extracted from seed pomace of *Camellia oleifera* Abel ameliorates DNCB-induced atopic dermatitis-like symptoms in BALB/c mice, *J. Funct. Foods* 91 (2022) 105001. <https://doi.org/10.1016/j.jff.2022.105001>.
- [25] E.Y. Kim, S. Hong, J.H. Kim, et al., Effects of chloroform fraction of *Fritillariae Thunbergii* Bulbus on atopic symptoms in a DNCB-induced atopic dermatitis-like skin lesion model and *in vitro* models, *J. Ethnopharmacol.* 281 (2021) 114453. <https://doi.org/10.1016/j.jep.2021.114453>.
- [26] Y.S. Lee, H.W. Ryu, W.K. Yang, et al., A combination of *Olea europaea* leaf extract and *Spirodela polyrhiza* extract alleviates atopic dermatitis by modulating immune balance and skin barrier function in a 1-chloro-2,4-dinitrobenzene-induced murine model, *Phytomedicine* 82 (2021) 153407. <https://doi.org/10.1016/j.phymed.2020.153407>.
- [27] M. Yamamoto, T. Haruna, K. Yasui, et al., A novel atopic dermatitis model induced by topical application with dermatophagoides farinae extract in NC/Nga mice, *Allergol. Int.* 56 (2007) 139-148. <https://doi.org/10.2332/allergolint.O-06-458>.
- [28] C.H. Park, S.Y. Min, H.W. Yu, et al., Effects of apigenin on RBL-2H3, RAW264.7, and HaCaT cells: anti-allergic, anti-inflammatory, and skin-protective activities, *Int. J. Mol. Sci.* 21 (2020). <https://doi.org/10.3390/ijms21134620>.
- [29] M. Gatzka, Skin under Tnf influence: how regulatory T cells work against macrophages in psoriasis, *J. Pathol.* 241 (2017) 3-5. <https://doi.org/10.1002/path.4820>.
- [30] M.L. Cuellar-Núñez, E.G. De Mejia, G. Loarca-Piña, *Moringa oleifera* leaves alleviated inflammation through downregulation of IL-2, IL-6, and TNF- α in a colitis-associated colorectal cancer model, *Food Res. Int.* 144 (2021) 110318. <https://doi.org/10.1016/j.foodres.2021.110318>.
- [31] E. Song, J. Hwang, J. Choi, et al., MON-LB675: *Dracocephalum moldavica* L. seed extracts ameliorate photo-aging and wound healing in HaCaT cell and 3D skin model, *Clin. Nutr.* 38 (2019) S310. [https://doi.org/10.1016/S0261-5614\(19\)32130-2](https://doi.org/10.1016/S0261-5614(19)32130-2).
- [32] Y.W. Liu, Y. Liu, C.C. Zeng, et al., Concentrated growth factor promotes wound healing potential of HaCaT cells by activating the ras signaling pathway, *Front. Biosci.-Landmark* 27 (2022) 319. <https://doi.org/10.31083/j.fbl2712319>.
- [33] C.Y. Ma, N. He, Y.Y. Zhao, et al., Antimicrobial mechanism of hydroquinone, *Appl. Biochem. Biotechnol.* 189 (2019) 1291-1303. <https://doi.org/10.1007/s12010-019-03067-1>.
- [34] I. Kim, Y.K. Park, S.H. Song, et al., Akt1 is involved in tubular apoptosis and inflammatory response during renal ischemia-reperfusion injury, *Mol. Biol. Rep.* 47 (2020) 9511-9520. <https://doi.org/10.1007/s11033-020-06021-1>.
- [35] A. Elkamhawy, A.H.E. Hassan, S. Paik, et al., EGFR inhibitors from cancer to inflammation: discovery of 4-fluoro-N-(4-(3-(trifluoromethyl)phenoxy)pyrimidin-5-yl)benzamide as a novel anti-inflammatory EGFR inhibitor, *Bioorg. Chem.* 86 (2019) 112-118. <https://doi.org/10.1016/j.bioorg.2019.01.017>.
- [36] W.C. Lv, G.Q. Ren, K. Shimizu, et al., Mogroside IIE, an *in vivo* metabolite of sweet agent, alleviates acute lung injury via Pla2g2a-EGFR inhibition, *Food Sci. Hum. Wellness* 13 (2024) 299-312.
- [37] C.A.R.A. Costa, A.E.V. Quaglio, L.C.D. Stasi, *Pfaffia paniculata* (Brazilian ginseng) extract modulates MAPK and mucin pathways in intestinal inflammation, *J. Ethnopharmacol.* 213 (2018) 21-25. <https://doi.org/10.1016/j.jep.2017.10.009>.
- [38] M. Djouina, C. Waxin, S. Caboche, et al., Low dose dietary contamination with deoxynivalenol mycotoxin exacerbates enteritis and colorectal cancer in mice, *Sci. Total Environ.* 900 (2023) 165722. <https://doi.org/10.1016/j.scitotenv.2023.165722>.
- [39] G. Chen, C.H. Lv, Q. Nie, et al., Essential oil of *Matricaria chamomilla* alleviate psoriatic-like skin inflammation by inhibiting PI3K/Akt/mTOR and p38MAPK signaling pathway, *Clin. Cosmet. Inv. Derm.* 17 (2024) 59-77. <https://doi.org/10.2147/CCID.S445008>.
- [40] S.S. Zhang, D. Feng, J.Z. An, et al., Total glycosides of *Cistanche deserticola* attenuates DSS-induced inflammatory bowel disease by regulating intestinal environmental homeostasis, *Food Med. Homol.* 1 (2024) 9420048. <https://doi.org/10.26599/FMH.2025.9420048>.
- [41] C. Lu, S.J. Deng, Y.J. Liu, et al., Inhibition of macrophage MAPK/NF- κ B pathway and Th2 axis by mangiferin ameliorates MC903-induced atopic dermatitis, *Int. Immunopharmacol.* 133 (2024) 112038. <https://doi.org/10.1016/j.intimp.2024.112038>.
- [42] M.D. Howell, B.E. Kim, M. Boguniewicz, et al., Modulation of flaggrin by Th2 cytokines in the skin of atopic dermatitis (AD), *J. Allergy. Clin. Immun.* 119 (2007) S283. <https://doi.org/10.1016/j.jaci.2006.12.479>.
- [43] Y.F. Liu, N. Ling, B. Zhang, et al., Flavonoid-rich mulberry leaf extract modulate lipid metabolism, antioxidant capacity, and gut microbiota in high-fat diet-induced obesity: potential roles of FGF21 and SOCS2, *Food Med. Homol.* 1 (2024) 9420016. <https://doi.org/10.26599/FMH.2024.9420016>.
- [44] X. Guo, J.Y. Ji, J.K. Zhang, et al., Anti-inflammatory and osteoprotective effects of Chikusetsusaponin IVa on rheumatoid arthritis via the JAK/STAT signaling pathway, *Phytomedicine* 93 (2021) 153801. <https://doi.org/10.1016/j.phymed.2021.153801>.
- [45] H.S. Jung, Y.J. Park, B.H. Gu, et al., Coumarin derivatives ameliorate the intestinal inflammation and pathogenic gut microbiome changes in the model of infectious colitis through antibacterial activity, *Front. Cell Infect. Mi.* 14 (2024) 1362773. <https://doi.org/10.3389/fcimb.2024.1362773>.