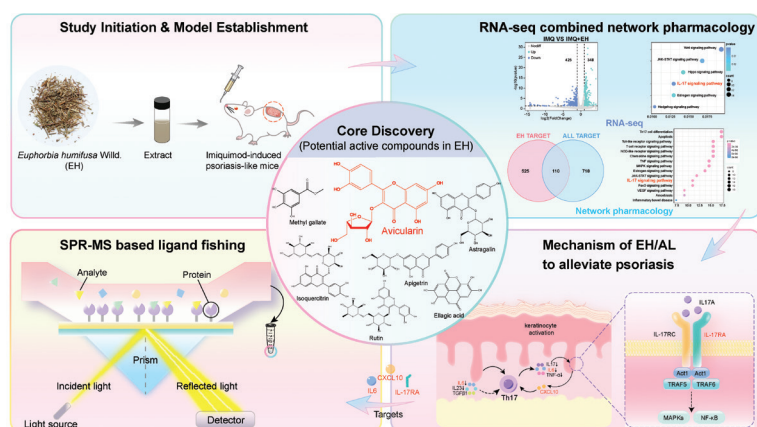


# Network pharmacology-guided discovery of avicularin from *Euphorbia humifusa* Willd. targeting IL-17 signaling in psoriasis

## Graphical abstract



## Authors

Qianqian Zhou, Zhe Wang, Yuanxiang Wang, Jiawei Guan, Linyun He, Jiwen Yang, Yaping Chi, Li Zhao, Bo Zhang, Kewu Zeng, Hua Zhang and Bo Han

## Correspondence

zh\_pha@shzu.edu.cn (H. Zhang);  
hanbo@shzu.edu.cn (B. Han)

## In brief

This study uses an integrated platform of network pharmacology, RNA-seq, and SPR-MS to discover avicularin from *Euphorbia humifusa* Willd. as a novel IL-17RA inhibitor. Experimental evidence demonstrates that avicularin directly binds IL-17RA and functionally inhibits the IL-17 signaling pathway, significantly ameliorating psoriatic dermatitis *in vivo*.

## Highlights

- **Novel Target Identification:** This study provides novel experimental evidence that avicularin (AL) directly binds IL-17RA and functionally inhibits the IL-17 signaling pathway, thus uncovering a previously unknown and specific mechanism underlying its anti-psoriatic activity.
- **Anti-psoriatic Efficacy Validation:** AL is newly demonstrated to exert potent anti-psoriatic effects *in vivo*, by significantly ameliorating psoriatic dermatitis, and suppressing key pathogenic mediators, including IL-17RA, IL-6, and CXCL10.
- **Integrated Discovery Platform:** This innovative strategy integrates network pharmacology, RNA-seq, and SPR-MS for screening active compounds with anti-psoriatic activity from *Euphorbia humifusa* Willd.

## Research Article

# Network pharmacology-guided discovery of avicularin from *Euphorbia humifusa* Willd. targeting IL-17 signaling in psoriasis

Qianqian Zhou<sup>a,1</sup>, Zhe Wang<sup>a,1</sup>, Yuanxiang Wang<sup>a</sup>, Jiawei Guan<sup>a</sup>, Linyun He<sup>a</sup>, Jiwen Yang<sup>a</sup>, Yaping Chi<sup>a</sup>, Li Zhao<sup>a</sup>, Bo Zhang<sup>a</sup>, Kewu Zeng<sup>b</sup>, Hua Zhang<sup>a,\*</sup> and Bo Han<sup>a,\*</sup>

<sup>a</sup>School of Pharmacy/Institute for Safflower Industry Research/Key Laboratory of Xinjiang Phytomedicine Resource and Utilization, Ministry of Education, Shihezi University, Shihezi 832002, China

<sup>b</sup>State Key Laboratory of Natural and Biomimetic Drugs, School of Pharmaceutical Sciences, Peking University, Beijing 100191, China

<sup>1</sup>These authors contributed equally to this article.

\*Correspondence: zh\_pha@shzu.edu.cn (H. Zhang); hanbo@shzu.edu.cn (B. Han)

Received: 27 August 2025; Revised: 28 November 2025; Accepted: 24 December 2025

Published online: 14 March 2026

DOI 10.15212/AMM-2025-0058

## ABSTRACT

Despite therapeutic advances, long-term psoriasis management remains challenged by cost, immunogenicity, and administration barriers. Although the anti-inflammatory properties of avicularin (AL) are recognized, its direct targeting of IL-17RA and its therapeutic efficacy in psoriasis remain unreported. Here, we established the efficacy of *Euphorbia humifusa* Willd. (EH) in a psoriasis mouse model, then used an integrated strategy combining network pharmacology, RNA-seq, and surface plasmon resonance-mass spectrometry (SPR-MS) to assess its pharmacodynamic material basis. This systematic approach identified the IL-17 pathway as the primary mechanism, and IL-6, CXCL10, and IL-17RA as key targets. On the basis of these key targets, SPR-MS screened seven candidate compounds from EH, among which AL was confirmed as a key IL-17RA-targeting bioactive compound. Subsequent validation demonstrated that AL dose-dependently ameliorated psoriatic lesions and downregulated key IL-17 pathway effectors, including IL-17RA, IL-6, and CXCL10 ( $p < 0.05$ ). Direct binding between AL and IL-17RA was subsequently confirmed through bio-layer interferometry, SPR-MS re-screening, and molecular docking. Our findings not only establish AL as a promising oral IL-17RA-targeted agent but also delineate a robust strategy that may aid in the systematic discovery of active natural compounds.

**Keywords:** Psoriasis, *Euphorbia humifusa* Willd., Network pharmacology, RNA-seq, SPR-MS, Avicularin

## 1. INTRODUCTION

Psoriasis is a chronic, immune-mediated, inflammatory skin disease that affects 2–3% of the global population [1]. This disease is associated with comorbidities such as psoriatic arthritis and cardiovascular disease, which decrease life expectancy by ~5 years [2]. The pathogenesis of psoriasis involves communication among various cytokines, chemokines, and other mediators of dendritic cells, Th17 cells, and keratinocytes; this communication induces skin immune cell infiltration and hyperproliferation of keratinocytes; leads to the development of typical skin lesion symptoms (e.g., interleukin (IL)-17 and TNF- $\alpha$ ); and drives keratinocyte hyperproliferation [3–5]. Currently approved biologics targeting IL-17A, IL-17RA, TNF- $\alpha$ , and IL-12/IL-23 are widely used

to manage moderate-to-severe psoriasis and have demonstrated significant efficacy in clinical practice [6]. Secukinumab, a first-line biologic recommended by the American Academy of Dermatology and the European Academy of Dermatology and Venereology guidelines, via intravenous or subcutaneous administration, effectively treats moderate-to-severe psoriasis by inhibiting IL-17A production [7]. Given concerns regarding the affordability, potential iatrogenic immunosuppression, and inconvenient administration modes of biologics, safe and effective alternative therapies for psoriasis are urgently needed [8].

*Euphorbia humifusa* Willd. (EH), a traditional Chinese medicine historically used for inflammatory skin disorders, is the principal component of Baixuan Xiatare tablet (BXXTR), a prescription for the treatment of plaque

psoriasis [9-11]. Additionally, the aqueous extract of EH has been experimentally shown to ameliorate imiquimod (IMQ)-induced psoriasiform skin lesions [11]. Phytochemical investigations have identified multiple classes of bioactive constituents in EH, including flavonoids, terpenoids, alkaloids, and polyphenols [12, 13], thus underscoring its potential as a valuable source for anti-psoriatic drug discovery. Among these, the flavonoid glycoside avicularin (AL) has demonstrated broad anti-inflammatory activity in various disease models, primarily through modulation of signaling pathways including NF- $\kappa$ B [14], MAPK [15], and NLRP3 inflammasome activation [16]. Nevertheless, its specific role in psoriasis pathogenesis, particularly its potential to directly target IL-17RA, a key signaling node in psoriatic immunity, has remained unclear. Given the chemical complexity of EH, efficient identification of its anti-psoriatic constituents is necessary to clarify its pharmacodynamic material basis.

Network pharmacology is a powerful approach for studying natural product-disease interactions yet is limited by database inaccuracy and static pathway representations [17-21]. RNA sequencing (RNA-seq) can supplement network pharmacology by identifying differentially expressed genes (DEGs) after treatment, thus offering dynamic mechanistic insights. For example, Li et al., by integrating RNA-seq with network pharmacology, have identified key DEGs and mechanisms through which acetylcorynoline alleviates inflammatory lung damage [22]. Wang et al. have identified the key targets and pathways underlying the analgesic effects of Shentong Zhuyu decoction through this combined approach [23]. Because RNA-seq provides real-world gene regulation data, it can validate and refine network pharmacology predictions, and consequently increase the reliability of the results [24]. Integrating these methods offers a robust framework for elucidating natural product mechanisms and addresses current limitations in target-pathway analysis.

Surface plasmon resonance-mass spectrometry (SPR-MS)-based ligand fishing enables direct, high-throughput screening of active compounds from natural products [25, 26]. Current SPR-MS methods typically preselect known disease targets but potentially overlook the multitarget nature of natural products [27, 28]. Without considering the actual intervention mechanisms of natural products, SPR-MS can introduce bias and miss key therapeutic mechanisms. A promising solution combines network pharmacology with RNA-seq to identify biologically relevant targets for SPR-MS screening. For example, Zeng et al. have used RNA-seq to identify TGF- $\beta$ 1 as an SPR bait and have successfully screened *przewalskin* from *Salvia miltiorrhiza* Bunge for pulmonary fibrosis alleviation [29]. These findings demonstrate that the integration of network pharmacology with RNA-seq can identify valuable targets for ligand fishing technology by elucidating

the mechanisms of natural products, thereby addressing the limitations of existing SPR-MS technologies in target selection.

To systematically investigate the anti-psoriatic basis of EH, we first used an approach integrating network pharmacology and RNA-seq, which convergently identified the IL-17 signaling pathway as the core mechanism. Subsequently, SPR-MS screening targeting IL-17RA and other core targets identified AL as the key active compound. Although AL's broad anti-inflammatory role was documented, its specific function in psoriasis was unclear. We validated that AL significantly alleviated psoriatic inflammation in a mouse model and directly downregulated the IL-17RA pathway. Our findings therefore redefine AL as a direct IL-17RA inhibitor, and suggest that, beyond its role as a general anti-inflammatory compound, it might serve as a candidate for targeted psoriasis therapy.

## 2. METHODS

### 2.1 Reagents

The materials and reagents used for SPR assays, including CM5 chips, N-hydroxysuccinimide, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC), phosphate-buffered solution (PBS), and ethanolamine, were obtained from GE Healthcare (Shanghai, China). Methanol and acetonitrile (optima grade) were purchased from Fisher Chemicals (Hampton, NH, USA), and 5% IMQ cream (5% concentration) was obtained from Sichuan Mingxin Pharmaceutical Co., Ltd. (Sichuan, China). Methotrexate (MTX) tablets were supplied by SPH Sine Pharmaceutical Laboratories Co., Ltd. (Shanghai, China). BXXTR was supplied by Yinduolan Pharmaceutical Industry Medicine Co., Ltd. (Urumqi, China). AL, isoquercitrin, methyl gallate, astragaloside, gallic acid, catechin, myricetin, ellagic acid, rutin, apigenin, luteolin, quercetin, naringenin, kaempferol, and isorhamnetin were obtained from Yuanye Bio-Technology Co., Ltd. (Shanghai, China). IL-17RA, IL-6, and CXCL10 were purchased from Novoprotein (Jiangsu, China). Anti-IL-17RA (cat. No. ab318130) and anti-GAPDH (cat. No. ab8245) were purchased from Abcam (Cambridge, UK). Anti-IL-6 (cat. No. #12912) was purchased from Cell Signaling Technology (Boston, USA). Anti-CXCL10 (cat. No. DF6417) was purchased from Affinity Biosciences (Jiangsu, China). Affinity-purified goat anti-rabbit IgG (H + L) (cat. No. JAC-111-035-003) and goat anti-mouse IgG (H + L) (cat. No. JAC-115-035-003) were purchased from Jackson ImmunoResearch Laboratories (West Grove, PA, USA).

### 2.2 Preparation of EH extract

EH was obtained from Kanghe Traditional Chinese Medicine Technology Co., Ltd. (Anhui, China) and was identified as *Euphorbia humifusa* Willd. by Professor Bo Han. The voucher specimen (No. 20191220) was archived in the Key Laboratory of Xinjiang Phytomedicine

## Research Article

Resource and Utilization (School of Pharmacy, Shihezi University). The EH was prepared according to the preparation process for BXXTR. Briefly, with 70% ethanol (v/v) as the solvent, 54.00 g EH was precisely weighed in a 1:4 (w/v) ratio for reflux extraction, which was conducted three times for 1 h each. The residue was collected for decoction with water for 1 h, and this process was repeated three times. The filtrates were combined and concentrated to a 300 mL stock solution.

### 2.3 IMQ-induced psoriasis-like mouse model and intervention

Male BALB/c mice (6–8 weeks old, 18–22 g) were obtained from the Animal Experimental Center of Xinjiang Medical University (Urumqi, China) (certificate number SCXK (Xin) 2023–0002). The mice were routinely housed for 1 week with free access to water and food, under light and dark conditions alternating every 12 h. After a 7-day acclimation period, the mice were randomly assigned to eight groups ( $n = 6$ ): control (normal saline), model (IMQ), low-dose EH (IMQ + EH-L, 200 mg/kg/0.2 mL, i.g.), moderate-dose EH (IMQ + EH-M, 400 mg/kg/0.2 mL, i.g.), high-dose EH (IMQ + EH-H, 800 mg/kg/0.2 mL, i.g.), BXXTR (IMQ + BXXTR/0.2 mL, 1 g/kg, i.g.), MTX (IMQ + MTX/0.2 mL, 1 mg/kg, i.g.), and EH (EH, 200 mg/kg/0.2 mL, i.g.). In line with most prior research investigating natural products for psoriasis mitigation, we used MTX, the primary oral systemic treatment for psoriasis, as a positive control [30]. All mice except those in the control and EH groups received daily topical application of 62.5 mg of 5% IMQ cream on their shaved backs and ears for seven consecutive days, to establish a psoriasis-like mouse model. In parallel, an equal amount of petrolatum was applied to the control and EH groups. The treatments for all groups were started 6 hours after model establishment and continued for seven consecutive days. During the intervention period, the Psoriasis Area and Severity Index (PASI) scores and ear thickness were recorded daily. On day 8 after the drug intervention in each group, all mice were euthanized by isoflurane inhalation, and skin, blood, ear, and spleen samples were promptly collected for subsequent analysis.

### 2.4 RNA sequencing

Skin samples from IMQ-induced psoriasis model mice with or without EH-H treatment ( $n = 5$ ) were collected on day 8. RNA isolation, library construction, and RNA-seq were performed by Shanghai Personal Biotechnology Co., Ltd. DEGs were screened on the basis of a threshold of  $|\log_2 \text{ fold change}| > 1$  and  $p < 0.05$ . Because proteins achieve their biological activity through protein-protein interaction (PPI), we reasoned that the construction of PPI networks would help elucidate the mechanisms of EH. DEGs were uploaded into the STRING database (version 12.0) to establish a PPI network for *Homo sapiens*. Network visualization was performed with Cytoscape (v3.9.1). The DAVID (version 2021) database was used

to conduct Gene Ontology (GO) analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis of the DEGs, and the enrichment results were visualized with the SRplot platform [31]. The Molecular Complex Detection 2.0.2 (MCODE, version 2.0.2) Cytoscape plug-in was used to extract core gene modules. Core genes were extracted from the core network through topological analysis with CytoNCA (version 2.1.0), according to the selection criteria of betweenness centrality (BC), closeness centrality (CC), and degree (De) values all exceeding the mean.

### 2.5 UHPLC-Q Exactive Orbitrap analysis of EH extract

Stock EH extract was diluted with 70% (v/v) aqueous ethanol to achieve a concentration of 50 mg/mL. The supernatant was separated by centrifugation at  $6797 \times g$  for 15 min. A Dionex UltiMate 3000 UHPLC system combined with a Q-Exactive mass spectrometer (Thermo Fisher Scientific, Carlsbad, CA, USA) was used to identify the material basis of EH. Chromatographic separation was performed with an Acquity UPLC BEH C18 column ( $\Phi 2.1 \text{ mm} \times 100 \text{ mm}$ ,  $1.7 \mu\text{m}$ ) at  $35^\circ\text{C}$ . The mobile phase consisted of water containing 0.1% formic acid (A) and acetonitrile (B). The gradient elution program for chromatographic separation was as follows: 0 min, 5% B; 1 min, 5% B; 20 min, 98% B; 25 min, 98% B; 25.2 min, 10% B; and 28 min, 10% B. MS analysis was conducted in positive and negative ionization modes, by using a heated electrospray ionization source, within a scan range of  $m/z$  90–1300 at a resolution of 70,000. The results were analyzed in Compound Discoverer software version 3.3, and target compounds were identified with the mzCloud secondary database.

### 2.6 Network pharmacology analysis

The Swiss Target Prediction database (version 2023.12, probability  $> 0$ ) was used to predict targets of the compounds identified by LC-MS. Potential psoriasis therapeutic targets were obtained from various databases, including DrugBank (version 5.1.12), GeneCards (v4.13, relevance score greater than or equal to the median), PharmGKB (2024.2), DisGeNET (v7.0, score greater than or equal to the median), Online Mendelian Inheritance in Man (OMIM 2024), and Therapeutic Target Database (TTD, version 2023), with the keyword “psoriasis.” The overlapping targets between the psoriasis-associated targets and EH components were considered potential candidates for EH intervention. The STRING database was then used to conduct PPI analysis of the overlapping targets (minimum interaction score  $> 0.4$ ), and Cytoscape was used to visualize the PPI network. According to the methods in Section 2.4, module and centrality analyses of the PPI network and core target identification were performed via the MCODE and CytoNCA plugins. The DAVID database was then used to identify enriched pathways associated with the core

network or genes. The enrichment results were visualized with SRplot (version 2024), a data visualization and graphing platform [31].

## 2.7 SPR-MS analysis based on key targets to screen active compounds

All SPR analyses were conducted on a Biacore T200 system (GE Healthcare, Sweden). Human recombinant proteins (IL-17RA, IL-6, and CXCL10) were dissolved in deionized water to achieve a concentration of 1 mg/mL, which was used for storage. To identify the optimal pH for protein conjugation, we diluted protein stock solutions to 50 µg/mL with four buffers at pH values of 4, 4.5, 5, and 5.5. Flow cell 1 of the CM5 chips was used as a blank, and the remaining three flow cells served as detection flow cells. The protein stock solutions were diluted to 50 µg/mL with the optimally conjugated pH sodium acetate buffer. The proteins were immobilized on CM5 chips via an amine coupling procedure after the surface was activated with 200 µL N-hydroxysuccinimide/EDC mixture. Unbound carboxyl groups on the CM5 chips were blocked with ethanolamine. The Biacore T200 system was used to conduct the injection and recovery procedure to screen for potential active compounds in EH as follows: 100 µg/mL treated EH extract was injected over the sensor surface, and compounds that bound to the protein remained, whereas nonbinding compounds were eluted with PBS. After 15 injection and recovery cycles, the bound compounds were dissociated with 0.5% formic acid, and 50 mM ammonium bicarbonate was added to obtain recovery solutions. The recovery solutions were concentrated under nitrogen, resuspended in 100 µL 80% methanol, and then centrifuged at 6797×g for 15 min for LC-MS.

## 2.8 Pathological skin observation

Mouse skin lesion samples were fixed in 4% neutral paraformaldehyde for 24 h, then embedded in paraffin. Paraffin-embedded skin lesion samples were sectioned into 3-µm slices and mounted on slides. The sections were subsequently stained with hematoxylin and eosin (H&E). Image-Pro Plus 6.0 software (Media Cybernetics, MD, United States) was used to quantify epidermal hyperplasia to measure the pixel area of the epidermis. Skin lesion sections were subjected to immunofluorescence (IF) staining by incubation with anti-Ki67 at 4°C overnight. The skin lesion sections were then incubated with goat anti-mouse IgG (SeraCare 5220-0341, 1:400) for 50 min at room temperature. Subsequently, the skin lesion sections were mounted with DAPI Fluoromount-G for immunohistochemistry (IHC) analysis. The sections were stained overnight at 4°C with anti-F4/80 (CST 70076, 1:300), then incubated with secondary antibodies. The signal was visualized with DAB substrate.

## 2.9 Enzyme-linked immunosorbent assays

Serum was obtained from mouse blood by centrifugation at 956×g for 10 min at 4°C. The serum levels

of IL-6, CXCL10, IL-17A, IL-17B, IL-17F, TNF- $\alpha$ , IL-23, and IFN- $\gamma$ , key inflammatory cytokines in the IL-17 signaling pathway, were quantified with enzyme-linked immunosorbent assay (ELISA) kits (Lunchangshuo Biotech, Xiamen, China).

## 2.10 Molecular docking validation

Molecular docking of candidate active compounds with key targets was performed. The 3D crystal structures of key targets were retrieved from the Protein Data Bank. Before docking, AutoDock Tools (version 1.5.6) was used to prepare the receptor protein to remove water molecules and salt ions, and add polar hydrogen atoms. The 3D structures of the active compounds were retrieved from the PubChem database, hydrogenated via AutoDock Tools, selected as ligands, and exported as pdbqt files. Molecular docking between the screened compounds and targets was conducted with AutoDock Vina (version: 1.2.3). The affinity (kcal/mol) obtained was used to validate the binding activity between the compounds and proteins. LigPlot<sup>+</sup> v.2.2 was used to analyze intermolecular interactions and their strengths in the protein-ligand complex, including 2D hydrogen bond interactions, hydrophobic contacts, and atom accessibility.

## 2.11 *In vivo* experimental validation of the activity of candidate compounds

An IMQ-induced psoriasis-like mouse model was established according to a previously described method to validate the activity of the screened compounds. Briefly, after the 7-day acclimation period, the mice were randomly divided into seven groups ( $n = 6$ ): control, model (IMQ), AL-H (IMQ + AL-H, 18 mg/kg, i.g.), AL-L (IMQ + AL-L, 9 mg/kg, i.g.), MG (IMQ + MG, 70 mg/kg, i.g.), AS (IMQ + AS, 16 mg/kg, i.g.), IQ (IMQ + IQ, 16 mg/kg, i.g.), and MTX (IMQ + MTX, 1 mg/kg, i.g.). All compounds were prepared with 10% PEG-400 as the solvent and administered daily for 7 consecutive days. Ear thickness and PASI scores were recorded daily. On day 8, all mice were euthanized with inhaled isoflurane, and skin, blood, ear, spleen, and other tissues were collected for subsequent analysis.

## 2.12 Western blotting

Skin tissue samples from each mouse group were weighed, homogenized, and lysed with RIPA lysis buffer (Beyotime, China, P0013D). After 5 min centrifugation (12,000×g, 4°C), the supernatants were collected, and the protein concentrations were quantified with a bicinchoninic acid protein assay kit (Beyotime, China). Protein samples were separated by electrophoresis and subsequently transferred to polyvinylidene fluoride membranes. The membranes were blocked with 5% skim milk and incubated overnight at 4°C with primary antibodies to IL-17RA, CXCL10, IL-6, and GAPDH. The membranes were subsequently incubated with the corresponding secondary antibodies for 1 h at room temperature. Blot images were captured with a QuickChem

## Research Article

5100 imaging system (Monad, China), and the densitometry of the immunoblots was quantified in ImageJ software (NIH, USA).

### 2.13 Bio-layer interferometry binding kinetics assays

Bio-layer interferometry (BLI) assays were performed with an Octet R8 (Sartorius, Germany) instrument. The recombinant protein was prepared in PBS at a concentration of 1 mg/mL. For establishment of the baseline, the biosensor was prewetted with PBST (containing 5% DMSO and 0.05% Tween 20), and the protein was directly immobilized on the biosensor surface in 96-well black F-bottom plates. The active compound was serially diluted to concentrations ranging from 1.95 to 62.50  $\mu$ M in PBST, with a final volume of 200  $\mu$ L per well. As a control, 200  $\mu$ L PBST (containing 5% DMSO and 0.02% Tween 20) was added to the wells. Baseline, association, and dissociation steps were performed, each lasting 60 seconds. The response (in nm) indicated the optical thickness on the biosensor layer. The equilibrium binding signal ( $R_{eq}$ ) was revealed when the curve flattened.  $K_d$  was calculated by fitting of the maximum BLI response at different concentrations to a sigmoidal curve. Brodalumab, an FDA-approved IL-17RA monoclonal antibody with established efficacy in psoriasis treatment, was selected as the positive control [32]. Rosiglitazone, a ligand of peroxisome proliferator-activated receptor  $\gamma$  (PPAR $\gamma$ ), was used as the negative control drug [33].

### 2.14 Statistical analysis

The data were analyzed statistically in SPSS Version 26.0 (IBM Corp., Armonk, NY, USA). All data are expressed as mean  $\pm$  standard deviation (SD). One-way analysis of variance was used for comparisons among multiple groups. The thresholds for statistical significance were as follows: \*\*\* $p < 0.001$ , \*\* $p < 0.01$ , and \* $p < 0.05$ . Before parametric tests, all experimental datasets were systematically assessed for normality with the Shapiro-Wilk test. For datasets exhibiting a non-normal distribution ( $p < 0.05$ ), normality was re-evaluated after Johnson transformation. Data that remained non-normally distributed after transformation were analyzed with non-parametric alternatives (Kruskal-Wallis test followed by Dunn's post hoc analysis) rather than parametric analysis of variance.

## 3. RESULTS

### 3.1 EH alleviates skin lesions in an IMQ-induced psoriasis-like mouse model

To evaluate the efficacy of BXXTR and the principal herbal component (i.e., EH) for treating psoriasis, we established an IMQ-induced psoriasis-like mouse model. The mice were treated with EH, BXXTR, or MTX (Figure 1A). IMQ-induced erythematous squamous

plaques were alleviated after treatment with various concentrations of EH (Figure 1B). The PASI score, which is frequently used to assess the severity of psoriatic lesions, indicated significant alleviation of scaling (Figure 1C) and erythema (Figure 1D) symptoms from IMQ-induced psoriasis-like lesions after treatment with various concentrations of EH. The total PASI score also showed consistent results (Figure 1E). The ability of EH to treat psoriasis was concentration-dependent. EH-H performed similarly to MTX, the positive control, in treating psoriasis. The EH-H intervention significantly inhibited IMQ-induced ear swelling ( $54.39\% \pm 8.39\%$ ) (Figure 1F). After the EH-H intervention, the spleen index (spleen/body weight ratio), which reflects inflammatory response severity, decreased by  $49.07\% \pm 8.01\%$  (Figure 1G). These findings indicated that EH might alleviate IMQ-induced immune responses in a concentration-dependent manner when the EH dose for BXXTR is consistent.

To further assess the effect of EH on histological changes in IMQ-induced psoriasis-like skin lesions, we performed H&E staining on skin samples from mice in each group. The skin of the IMQ groups presented significant epidermal layer thickening and rete ridges (Figure 1H). Statistical analyses further revealed that IMQ-induced epidermal thickening was inhibited by  $41.55\% \pm 14.00\%$  after EH-H intervention (Figure 1I). In addition, compared with MTX treatment, EH treatment significantly alleviated the symptoms of IMQ-induced epidermal hyperplasia and parakeratosis of the spinous layer in the dermis. IHC and IF staining revealed fewer F4/80 macrophages and lower abundance of the keratinocyte proliferation marker Ki67 in the EH-H treated and MTX groups than the IMQ group (Figure 1J). ELISA revealed that, compared with those in the IMQ group, the levels of IL-17A, IL-17B, IL-17F, IFN- $\gamma$ , IL-23, and TNF- $\alpha$  in the EH-H group were  $39.35\% \pm 14.35\%$ ,  $31.51\% \pm 6.23\%$ ,  $39.75\% \pm 6.29\%$ ,  $32.70\% \pm 14.96\%$ ,  $30.12\% \pm 15.09\%$ , and  $46.74\% \pm 9.98\%$  lower, respectively (Supplementary Figure 1). Overall, EH exerted a significant therapeutic effect in an IMQ-induced psoriasis-like mouse model by alleviating keratinocyte hyperproliferation and the inflammatory response.

### 3.2 Transcriptome sequencing analysis of the therapeutic effect of EH on psoriasis

To validate and complement the predictive results of network pharmacology, we conducted RNA-seq analysis on skin lesions from the IMQ and EH-H groups (Figure 2A). A total of 773 DEGs were screened, including 348 upregulated and 425 downregulated mRNAs, all of which were significant at  $p < 0.05$  and  $|\log_2 \text{fold change}| > 1$  (Figure 2B,C). The functional enrichment of DEGs was evaluated through GO annotation and KEGG pathway analyses. The top ten enriched GO terms were extracellular region, intermediate filament, intermediate filament

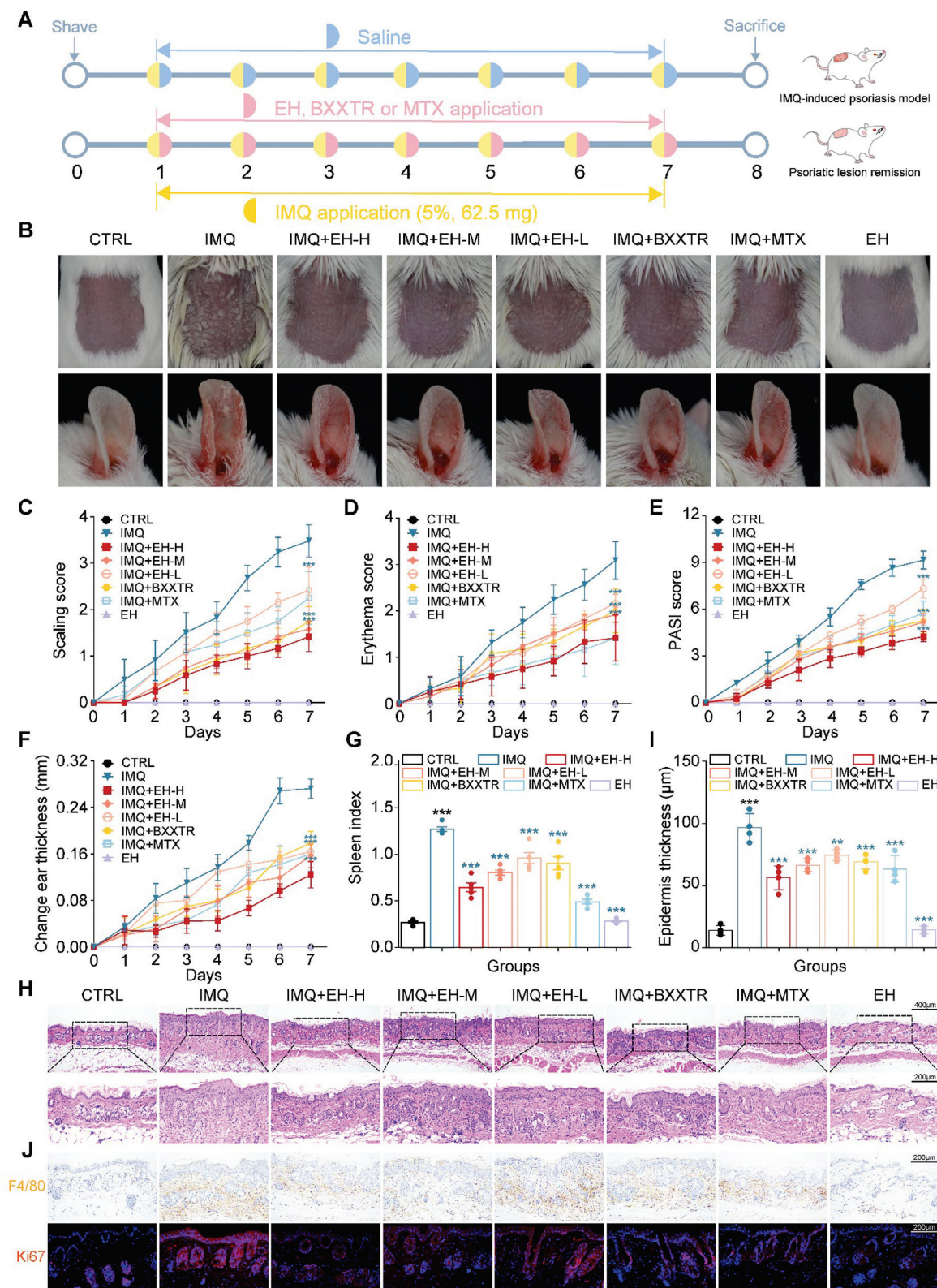


Figure 1 | EH alleviates IMQ-induced psoriasis *in vivo*.

## Research Article

(A) Schemes of the IMQ-induced psoriasis-like model. (B) Representative images of lesions on day 8. (C–F) Quantification of the scaling score, erythema score, PASI score, and changes in ear thickness.  $n = 6$  samples/group. Data are presented as mean  $\pm$  SD. (G) Spleen weight index, calculated as the ratio of spleen weight to body weight.  $n = 4$  samples/group. Data are presented as mean  $\pm$  SD. (H) H&E staining and (I) corresponding quantification. Scale bar = 400  $\mu$ m and 200  $\mu$ m.  $n = 4$ . Data are presented as mean  $\pm$  SD. (J) Immunohistochemistry and immunofluorescence staining of F4/80 or Ki67 in skin lesions at day 8 (scale bar = 200  $\mu$ m).

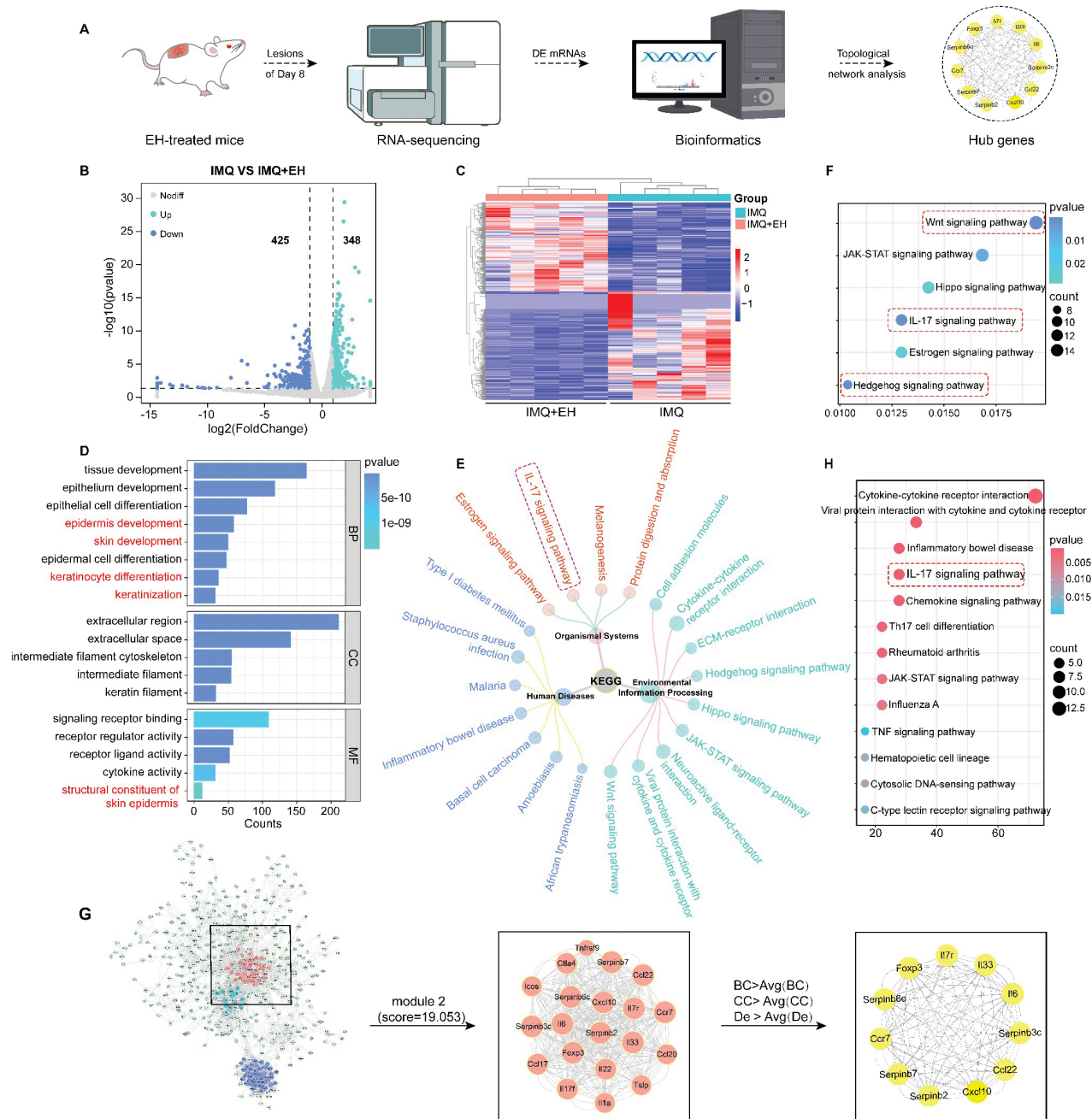


**Figure 2 | RNA-sequencing of skin lesions from the IMQ + EH-H (EH-H) group and IMQ group ( $n = 5$ ).**

(A) Schematic illustration of key target identification. (B, C) Volcano plots and heatmap of DEGs. (D) Representative entries for GO\_BP, GO\_MF, and GO\_CC. (E) KEGG secondary categorization of DEGs. (F) Representative KEGG signaling pathway entries. (G) PPI network of the DEGs. (H) KEGG analysis of the key network module.

cytoskeleton, keratinization, tissue development, epidermis development, epithelium development, epidermal cell differentiation, extracellular space, and keratin filament (Figure 2D). KEGG analysis revealed that pathways such as cytokine-cytokine receptor interaction, basal cell carcinoma, *Staphylococcus aureus* infection, inflammatory bowel disease, viral protein interaction with cytokine and cytokine receptor, Hedgehog signaling pathway, Wnt signaling pathway, IL-17 signaling pathway, malaria, and neuroactive ligand-receptor interactions showed significant differences between the IMQ group and the EH-H group (Figure 2E). In mice with EH-treated IMQ-induced psoriasis, Wnt signaling pathway, IL-17 signaling pathway, and Hedgehog signaling pathway expression in skin lesions was strongly affected (Figure 2F). These findings indicated that EH might potentially ameliorate psoriasis-like dermatitis by modulating skin development and inflammatory cascades.

To further identify the key genes involved in the amelioration of psoriatic dermatitis by EH, we constructed a PPI network containing 767 nodes and 2426 edges (Figure 2G). Three closely linked gene modules were extracted via the MCODE plug-in of Cytoscape. Module 1 (score = 25.778) contained 28 targets and 696 interaction pairs; module 2 (score = 19.053) contained 20 targets and 362 interaction pairs; and module 3 (score = 7.765) contained 18 targets and 132 interaction pairs (Supplementary Figure S2A). Enrichment analysis revealed that module 2 was associated with cytokine-cytokine receptor interaction, the IL-17 signaling pathway, the chemokine signaling pathway, Th17 cell differentiation, extracellular regions, extracellular spaces, inflammatory responses, and immune responses (Figure 2H, Supplementary Figure S2B). The targets in modules 1 and 3 were associated with skin development and melanin biosynthesis, respectively (Supplementary Figure S2C–F). Because of its close association with psoriasis pathogenesis, we focused on module 2 for topological network analysis to explore core genes. Using the CytoNCA plugin and topology parameters of BC > average (BC = 0.90), CC > average (CC = 0.96), and De > average (De = 36.20), we evaluated the regulatory relationships between the targets in module 2. A subnet consisting of 11 nodes and 110 edges was extracted, which provided valuable insights into the therapeutic mechanisms of EH. Notably, important inflammatory cytokines, such as CXCL10 and IL-6, in the IL-17 signaling pathway were included in this network; therefore, EH might treat psoriasis by modulating the IL-17 signaling pathway.

### 3.3 Identification of EH compounds through UHPLC-Q Exactive Orbitrap Analysis

To obtain an overview of the compounds contained in EH, we sought to identify the material basis of EH. The compounds in EH were analyzed with UHPLC-Q Exactive Orbitrap analysis coupled with the mzCloud mass spectral database. The chromatograms of EH in both negative and positive ion modes are shown in Supplementary

Figure S3A,B. A total of 82 compounds in EH were identified (Table 1). The material basis of EH was confirmed through a mixed standard solution containing 15 compounds (Supplementary Figure S3C). Further analysis revealed that flavonoids were the most abundant class in EH (31 identified compounds). Additionally, the results revealed 22 compounds classified as fatty acid derivatives, eight compounds classified as phenolic acids, four compounds classified as coumarins, three compounds classified as lignans, three compounds classified as steroids, and three compounds classified as sphingolipids (Supplementary Figure S4). UHPLC-Q Exactive Orbitrap analysis was used to identify the chemical composition of EH in preparation for subsequent network pharmacology analysis.

### 3.4 Network pharmacology integrated with RNA-seq analysis

A total of 828 psoriasis-associated targets were collected from the OMIM, DrugBank, GeneCards, DisGeNET, and PharmGKB databases after duplicate removal. A total of 82 compounds in EH identified by LC-MS (Table 1) were subjected to network pharmacology analysis, which identified 635 putative targets. By intersecting these targets with psoriasis-associated genes, we identified 110 overlapping drug-disease intersection targets (Figure 3A). A PPI network (109 nodes, 2306 edges) highlighted key targets with strong interactions, thus suggesting their potential roles as critical regulators in psoriasis pathophysiology (Figure 3B). Pathway enrichment analysis indicated that EH might alleviate psoriasis via the Toll-like receptor, TNF, IL-17, and JAK-STAT pathways, which are intricately involved in the inflammatory and immune responses that underlie psoriasis (Figure 3C). GO analysis further supported the role of EH in regulating signal transduction, inflammation, apoptosis, immune responses, and cell differentiation (Figure 3D). Cytoscape MCODE analysis revealed one significant functional module (33 targets and 906 interactions) (Figure 3E). With the screening criteria of BC > average (BC = 4.55), CC > average (CC = 0.89), and DE > average (DE = 55.00), CytoNCA identified 17 key targets from the module. These targets exhibited enrichment in IL-17 signaling, TNF signaling, and Th17 cell differentiation pathways (Figure 3F). GO analysis indicated that these targets are involved in negative regulation of gene expression, miRNA transcription, xenobiotic response, and transcription regulator complexes (Figure 3G). These findings suggested that EH might alleviate psoriasis-like lesions by influencing skin development and inflammatory responses, in agreement with the RNA-seq findings.

To address the inherent uncertainty in network pharmacology predictions due to database limitations, we integrated RNA-seq data to elucidate EH's mechanisms in alleviating psoriasis. Comparative analysis of the targets identified by network pharmacology and RNA-seq revealed five overlapping targets: G-protein coupled receptor 35 (GPR35), C-X-C motif chemokine receptor 2

**Table 1 |** Mass spectrometric parameters and fragment spectra of EH compounds.

| No. | $t_R$ (min) | Name                                   | Monitoring mode                     | Formula                                         | Error (ppm) | Calculated MW | Experimental mass ( <i>m/z</i> ) | MSMS data (+ or -) ( <i>m/z</i> )                                   | Classification |
|-----|-------------|----------------------------------------|-------------------------------------|-------------------------------------------------|-------------|---------------|----------------------------------|---------------------------------------------------------------------|----------------|
| 1*  | 1.43        | Gallic acid                            | [M-H] <sup>-</sup>                  | C <sub>7</sub> H <sub>6</sub> O <sub>5</sub>    | -8.69       | 170.0201      | 169.0128                         | 125.0228, 169.0129, 97.0277, 69.0329                                | Phenolic acids |
| 2   | 2.39        | (-)-Epigallocatechin                   | [M+H] <sup>+</sup>                  | C <sub>15</sub> H <sub>14</sub> O <sub>7</sub>  | 5.73        | 306.0740      | 307.0830                         | 139.0400, 163.0402, 181.0508, 169.0507, 223.0607                    | Phenolic acids |
| 3   | 2.63        | Coumaric acid                          | [M+H-H <sub>2</sub> O] <sup>+</sup> | C <sub>9</sub> H <sub>8</sub> O <sub>3</sub>    | 5.74        | 164.0437      | 147.0449                         | 147.0452, 119.0499, 91.0553                                         | Phenolic acids |
| 4   | 3.61        | Ferulic acid                           | [M+H] <sup>+</sup>                  | C <sub>10</sub> H <sub>10</sub> O <sub>4</sub>  | 5.71        | 194.0579      | 195.0663                         | 177.0558, 145.0296, 194.0392, 117.0341                              | Phenolic acids |
| 5*  | 4.37        | Catechin                               | [M-H] <sup>-</sup>                  | C <sub>15</sub> H <sub>14</sub> O <sub>6</sub>  | -0.47       | 290.0789      | 289.0716                         | 289.0703, 245.0826, 109.0278, 125.0228                              | Phenolic acids |
|     | 4.52        |                                        | [M+H] <sup>+</sup>                  |                                                 | 5.79        |               | 291.0880                         | 289.0715, 109.0278, 245.0813, 125.0228, 203.0703                    |                |
| 6*  | 4.50        | Methyl gallate                         | [M-H] <sup>-</sup>                  | C <sub>8</sub> H <sub>8</sub> O <sub>5</sub>    | -8.18       | 184.0372      | 183.0284                         | 183.0288, 168.0055, 124.0145, 111.0066                              | Phenolic acids |
| 7   | 4.62        | 6-Methylcoumarin                       | [M+H] <sup>+</sup>                  | C <sub>10</sub> H <sub>8</sub> O <sub>2</sub>   | 5.55        | 160.0524      | 161.0606                         | 139.0403, 123.0451, 147.0452, 165.0557, 291.0875, 207.0676          | Coumarins      |
| 8   | 4.62        | Syringin                               | [M+NH <sub>4</sub> ] <sup>+</sup>   | C <sub>17</sub> H <sub>24</sub> O <sub>9</sub>  | 6.52        | 372.1420      | 390.1784                         | 161.0603, 193.0865, 133.0658, 211.0969, 105.0710                    | Lignans        |
| 9   | 4.71        | Scopoletin                             | [M+H] <sup>+</sup>                  | C <sub>10</sub> H <sub>8</sub> O <sub>4</sub>   | 6.65        | 192.0435      | 193.0508                         | 193.0510, 133.0658, 161.0609, 105.0710                              | Coumarins      |
| 10  | 5.01        | Fraxidin                               | [M+H] <sup>+</sup>                  | C <sub>11</sub> H <sub>10</sub> O <sub>5</sub>  | 5.83        | 222.0528      | 223.0614                         | 223.0600, 208.0380, 190.0284, 163.0407                              | Coumarins      |
| 11  | 5.19        | Corilagin                              | [M+NH <sub>4</sub> ] <sup>+</sup>   | C <sub>27</sub> H <sub>22</sub> O <sub>18</sub> | 7.13        | 634.0851      | 652.1190                         | 277.0362, 465.0693, 303.0155, 259.0256, 127.0401                    | Tannins        |
| 12  | 5.60        | Fraxetin                               | [M-H] <sup>-</sup>                  | C <sub>10</sub> H <sub>8</sub> O <sub>5</sub>   | -4.50       | 208.0362      | 207.0290                         | 192.0055, 207.0292, 164.0105                                        | Coumarins      |
|     | 5.61        |                                        | [M+H] <sup>+</sup>                  |                                                 | 6.16        |               | 209.0457                         | 209.0444, 149.0234, 163.0389, 194.0221                              |                |
| 13* | 5.80        | Myricetin                              | [M+H] <sup>+</sup>                  | C <sub>15</sub> H <sub>10</sub> O <sub>8</sub>  | 5.62        | 318.0394      | 319.0466                         | 319.0469, 320.0504, 153.0193, 273.0415, 245.0458, 165.0193          | Flavonoids     |
| 14  | 5.83        | Myricetin 3-O-beta-D-galactopyranoside | [M-H] <sup>-</sup>                  | C <sub>21</sub> H <sub>20</sub> O <sub>13</sub> | -0.54       | 480.0901      | 479.0829                         | 316.0220, 479.0820, 271.0245                                        | Flavonoids     |
|     | 5.84        |                                        | [M+H] <sup>+</sup>                  |                                                 | 7.10        |               | 481.1011                         | 319.0470, 85.0295, 91.0401, 153.0192, 273.04156                     |                |
| 15* | 6.16        | Ellagic acid                           | [M-H] <sup>-</sup>                  | C <sub>14</sub> H <sub>6</sub> O <sub>8</sub>   | -1.96       | 302.0063      | 300.9984                         | 300.9977, 229.0138, 257.0089, 283.9958, 185.0225, 201.0179          | Phenolic acids |
| 16* | 6.18        | Rutin                                  | [M-H] <sup>-</sup>                  | C <sub>27</sub> H <sub>30</sub> O <sub>16</sub> | -0.08       | 610.1533      | 609.1461                         | 300.0270, 609.1459                                                  | Flavonoids     |
| 17  | 6.23        | Myricetin-3-xyloside                   | [M+H] <sup>+</sup>                  | C <sub>20</sub> H <sub>18</sub> O <sub>12</sub> | 5.76        | 450.0798      | 451.0897                         | 319.0481, 153.0198, 245.0476, 73.0293, 273.0401, 217.0504, 451.0898 | Flavonoids     |
|     | 6.26        |                                        | [M-H] <sup>-</sup>                  |                                                 | 0.56        |               | 449.0723                         | 316.0213, 449.0710, 271.0252, 287.0211                              |                |
| 18  | 6.28        | Kaempferol-3-O-glucuronoside           | [M+H] <sup>+</sup>                  | C <sub>21</sub> H <sub>18</sub> O <sub>12</sub> | 7.77        | 462.0798      | 463.0907                         | 287.0557, 463.0930                                                  | Flavonoids     |
| 19  | 6.28        | Scutellarin                            | [M+H] <sup>+</sup>                  | C <sub>21</sub> H <sub>18</sub> O <sub>12</sub> | 6.40        | 462.0828      | 463.0901                         | 287.0569, 288.0602, 463.0903                                        | Flavonoids     |
| 20* | 6.39        | Isoquercitrin                          | [M+H] <sup>+</sup>                  | C <sub>21</sub> H <sub>20</sub> O <sub>12</sub> | 6.11        | 464.0952      | 465.1056                         | 303.0527, 85.0297                                                   | Flavonoids     |

Table 1 | Continued

| No.  | $t_R$ (min) | Name                                                   | Monitoring mode                     | Formula                                         | Error (ppm) | Calculated MW | Experimental mass ( $m/z$ ) | MSMS data (+ or -) ( $m/z$ )                                       | Classification         |
|------|-------------|--------------------------------------------------------|-------------------------------------|-------------------------------------------------|-------------|---------------|-----------------------------|--------------------------------------------------------------------|------------------------|
| 6.45 |             |                                                        | [M-H] <sup>-</sup>                  |                                                 | -0.53       |               | 463.0880                    | 300.0271, 463.0883, 301.0344, 464.0924                             |                        |
| 21   | 6.52        | Cynaroside                                             | [M-H] <sup>-</sup>                  | C <sub>21</sub> H <sub>20</sub> O <sub>11</sub> | -0.12       | 448.1005      | 447.0932                    | 285.0400, 447.0932, 284.0323                                       | Flavonoids             |
| 22   | 6.69        | Nicotiflorin                                           | [M-H] <sup>-</sup>                  | C <sub>27</sub> H <sub>30</sub> O <sub>15</sub> | -0.09       | 594.1585      | 593.1513                    | 285.0399, 284.0323, 593.1514, 255.0296                             | Flavonoids             |
| 23   | 6.71        | Kaempferol-3-O-β-glucopyranosyl-7-O-α-rhamnopyranoside | [M+H] <sup>+</sup>                  | C <sub>27</sub> H <sub>30</sub> O <sub>15</sub> | 7.34        | 594.1628      | 595.1701                    | 287.0571, 85.0295, 97.0300                                         | Flavonoids             |
| 24   | 6.76        | Trifolin                                               | [M+H] <sup>+</sup>                  | C <sub>21</sub> H <sub>20</sub> O <sub>11</sub> | 6.22        | 448.1034      | 449.1106                    | 287.0571, 85.0295, 91.0403, 97.0295                                | Flavonoids             |
| 25*  | 6.83        | Avicularin                                             | [M-H] <sup>-</sup>                  | C <sub>20</sub> H <sub>18</sub> O <sub>11</sub> | -1.24       | 434.0849      | 433.0771                    | 301.0325, 433.0739, 151.0029, 271.0250, 255.0309, 73.0297, 57.0347 | Flavonoids             |
| 6.85 |             |                                                        | [M+H] <sup>+</sup>                  |                                                 | 6.13        |               | 435.0949                    | 303.0520, 133.0506, 85.0295, 73.0297, 57.0347                      |                        |
| 26   | 6.86        | Rhoifolin                                              | [M+H] <sup>+</sup>                  | C <sub>27</sub> H <sub>30</sub> O <sub>14</sub> | 7.08        | 578.1636      | 579.1749                    | 271.0619, 433.1162, 579.1767                                       | Flavonoids             |
| 6.88 |             |                                                        | [M-H] <sup>-</sup>                  |                                                 | -2.56       |               | 577.1548                    | 269.0469, 577.1533                                                 |                        |
| 27   | 6.90        | Cyanidin-3-O-galactoside                               | [M] <sup>+</sup>                    | C <sub>21</sub> H <sub>21</sub> O <sub>11</sub> | 6.15        | 449.1084      | 449.1106                    | 287.0558, 288.0597                                                 | Flavonoids             |
| 28*  | 6.93        | Astragalin                                             | [M-H] <sup>-</sup>                  | C <sub>21</sub> H <sub>20</sub> O <sub>11</sub> | -0.66       | 448.1003      | 447.0930                    | 285.0400, 447.0930, 284.0324, 255.0294                             | Flavonoids             |
| 29   | 7.05        | Isorhamnetin-3-O-glucoside                             | [M+H] <sup>+</sup>                  | C <sub>22</sub> H <sub>22</sub> O <sub>12</sub> | 6.88        | 478.1111      | 479.1217                    | 317.0681, 479.1239, 302.0445, 85.0298                              | Flavonoids             |
| 30*  | 7.13        | Apigetrin                                              | [M-H] <sup>-</sup>                  | C <sub>21</sub> H <sub>20</sub> O <sub>10</sub> | -0.80       | 432.1053      | 431.0980                    | 431.0980, 268.0374, 269.0440, 432.1013                             | Flavonoids             |
| 31   | 7.37        | Juglalin                                               | [M-H] <sup>-</sup>                  | C <sub>20</sub> H <sub>18</sub> O <sub>10</sub> | -1.64       | 418.0893      | 417.0820                    | 284.0323, 417.0826, 255.0294                                       | Flavonoids             |
| 32   | 7.64        | 4-Oxododecanedioic acid                                | [M+H] <sup>+</sup>                  | C <sub>12</sub> H <sub>20</sub> O <sub>5</sub>  | 5.91        | 244.1326      | 245.1398                    | 245.1399, 277.1291, 121.1022, 163.1128                             | Fatty acid derivatives |
| 33   | 7.75        | Alloxyptopine                                          | [M+H] <sup>+</sup>                  | C <sub>21</sub> H <sub>23</sub> NO <sub>5</sub> | 7.84        | 369.1576      | 370.1678                    | 370.1667, 188.0729, 290.0966, 352.1577                             | Alkaloids              |
| 34*  | 8.45        | Luteolin                                               | [M-H] <sup>-</sup>                  | C <sub>15</sub> H <sub>10</sub> O <sub>6</sub>  | -0.73       | 286.0475      | 285.0403                    | 285.0401, 286.0435, 133.0278                                       | Flavonoids             |
| 35*  | 8.51        | Quercetin                                              | [M-H] <sup>-</sup>                  | C <sub>15</sub> H <sub>10</sub> O <sub>7</sub>  | -1.58       | 302.0422      | 301.0349                    | 301.0349, 151.0022, 178.9973, 121.0278, 107.0122, 273.0396         | Flavonoids             |
| 36   | 8.54        | Nepetin                                                | [M+H] <sup>+</sup>                  | C <sub>16</sub> H <sub>12</sub> O <sub>7</sub>  | 5.22        | 316.0600      | 317.0672                    | 317.0676, 302.0440, 168.0064                                       | Flavonoids             |
| 37   | 9.05        | Matairesinol                                           | [M+H-H <sub>2</sub> O] <sup>+</sup> | C <sub>20</sub> H <sub>22</sub> O <sub>6</sub>  | 5.73        | 358.1437      | 341.1404                    | 137.0607, 359.1531                                                 | Lignans                |
| 38   | 9.34        | Apigenin                                               | [M-H] <sup>-</sup>                  | C <sub>15</sub> H <sub>10</sub> O <sub>5</sub>  | -0.84       | 270.0526      | 269.0453                    | 269.0452, 270.0486                                                 | Flavonoids             |
| 39*  | 9.38        | Naringenin                                             | [M-H] <sup>-</sup>                  | C <sub>15</sub> H <sub>12</sub> O <sub>5</sub>  | -0.93       | 272.0682      | 271.0609                    | 151.0028, 271.0590, 119.0481, 107.0120, 93.0326, 177.0179          | Flavonoids             |
| 40*  | 9.52        | Kaempferol                                             | [M+H] <sup>+</sup>                  | C <sub>15</sub> H <sub>10</sub> O <sub>6</sub>  | 5.26        | 286.0477      | 287.0565                    | 287.0568, 286.1155,                                                | Flavonoids             |

## Research Article

Table 1 | Continued

| No.  | $t_R$ (min) | Name                                                        | Monitoring mode                   | Formula                                                       | Error (ppm) | Calculated MW | Experimental mass ( $m/z$ ) | MSMS data (+ or -) ( $m/z$ )                      | Classification         |
|------|-------------|-------------------------------------------------------------|-----------------------------------|---------------------------------------------------------------|-------------|---------------|-----------------------------|---------------------------------------------------|------------------------|
| 9.54 |             |                                                             | [M-H] <sup>-</sup>                |                                                               | -1.62       |               | 285.0400                    | 285.0385, 151.0026, 229.0497, 185.0585, 169.0640, |                        |
| 41   | 9.54        | Isokaempferide                                              | [M-H] <sup>-</sup>                | C <sub>16</sub> H <sub>12</sub> O <sub>6</sub>                | -0.04       | 300.0635      | 299.0561                    | 299.0556, 284.0327, 256.0384, 277.0346            | Flavonoids             |
| 42   | 9.64        | (11E,15Z)-9,10,13-Trihydroxyoctadeca-11,15-dienoic acid     | [M+NH <sub>4</sub> ] <sup>+</sup> | C <sub>18</sub> H <sub>32</sub> O <sub>5</sub>                | 5.26        | 328.2267      | 346.2605                    | 173.1186, 155.1078, 109.1023, 275.2024            | Fatty acid derivatives |
| 43*  | 9.70        | Isorhamnetin                                                | [M-H] <sup>-</sup>                | C <sub>16</sub> H <sub>12</sub> O <sub>7</sub>                | -0.21       | 316.0583      | 315.0510                    | 315.0508, 300.0270, 316.0527                      | Flavonoids             |
| 44   | 10.41       | 12-Oxophytodienoic acid                                     | [M+H] <sup>+</sup>                | C <sub>18</sub> H <sub>28</sub> O <sub>3</sub>                | 4.67        | 292.2052      | 293.2125                    | 275.2024, 293.2122, 257.1911,                     | Fatty acid derivatives |
| 45   | 10.45       | 19-Norandrostenedione                                       | [M+H] <sup>+</sup>                | C <sub>18</sub> H <sub>24</sub> O <sub>2</sub>                | 5.04        | 272.1790      | 273.1863                    | 273.1868, 255.1769, 274.1901                      | Steroids               |
| 46   | 11.12       | Triphenylphosphine oxide                                    | [M+H] <sup>+</sup>                | C <sub>18</sub> H <sub>15</sub> OP                            | 5.69        | 278.0876      | 279.0949                    | 279.0962, 219.0571, 201.0487                      | Organophosphorus       |
| 47   | 11.44       | 5,2'-Dihydroxy-6,7,8,6'-tetramethoxyflavone                 | [M+H] <sup>+</sup>                | C <sub>19</sub> H <sub>18</sub> O <sub>8</sub>                | 5.65        | 374.1023      | 375.1096                    | 375.1102, 376.1149, 360.0859                      | Flavonoids             |
| 48   | 11.51       | (15Z)-9,12,13-Trihydroxy-15-octadecenoic acid               | [M-H] <sup>-</sup>                | C <sub>18</sub> H <sub>34</sub> O <sub>5</sub>                | -1.14       | 330.2403      | 329.233                     | 329.2330, 171.1014, 139.1112, 99.0797             | Fatty acid derivatives |
| 49   | 12.02       | Neobavaisoflavone                                           | [M+H] <sup>+</sup>                | C <sub>20</sub> H <sub>18</sub> O <sub>4</sub>                | 5.18        | 322.1222      | 323.1295                    | 323.1299, 267.0671, 255.0669, 69.0711, 239.0721   | Flavonoids             |
| 50   | 12.12       | Justicidin B                                                | [M+H] <sup>+</sup>                | C <sub>21</sub> H <sub>16</sub> O <sub>6</sub>                | 3.11        | 364.0947      | 365.1031                    | 365.1051, 321.1122, 335.0951, 303.0679, 291.1021  | Lignans                |
| 51   | 12.91       | Aurantiamide acetate                                        | [M+Na] <sup>+</sup>               | C <sub>27</sub> H <sub>28</sub> N <sub>2</sub> O <sub>4</sub> | 6.15        | 444.2049      | 467.1970                    | 467.1949, 105.0344, 407.1769, 224.1097            | Others                 |
| 52   | 13.02       | Corylin                                                     | [M+H] <sup>+</sup>                | C <sub>20</sub> H <sub>16</sub> O <sub>4</sub>                | 3.63        | 320.1049      | 321.1133                    | 321.1124, 137.0238, 306.0929, 303.1034            | Flavonoids             |
| 53   | 13.23       | Bis(4-ethylbenzylidene)sorbitol                             | [M+H] <sup>+</sup>                | C <sub>24</sub> H <sub>30</sub> O <sub>6</sub>                | 5.84        | 414.2067      | 415.2139                    | 119.0866, 135.0814, 415.2146                      | Others                 |
| 54   | 13.23       | (±)12(13)-DiHOME                                            | [M-H] <sup>-</sup>                | C <sub>18</sub> H <sub>34</sub> O <sub>4</sub>                | -1.26       | 314.2453      | 313.2380                    | 313.2380, 183.1377, 129.0906, 99.0797             | Fatty acid derivatives |
| 55   | 13.48       | (±)9(10)-DIHOME                                             | [M-H] <sup>-</sup>                | C <sub>18</sub> H <sub>34</sub> O <sub>4</sub>                | -1.27       | 314.2453      | 313.2380                    | 313.2381, 201.1121, 314.3410                      | Fatty acid derivatives |
| 56   | 13.56       | 2',6'-dihydroxy-4'-methoxydihydrochalcone                   | [M-H] <sup>-</sup>                | C <sub>16</sub> H <sub>16</sub> O <sub>4</sub>                | 0.06        | 272.1049      | 271.0976                    | 271.0971, 152.0094, 238.0638, 253.0871, 124.0145  | Flavonoids             |
| 57   | 13.65       | 17 $\alpha$ -Methyl-androstan-3-hydroxyimine-17 $\beta$ -ol | [M+H] <sup>+</sup>                | C <sub>20</sub> H <sub>33</sub> NO <sub>2</sub>               | -2.80       | 319.2502      | 320.2575                    | 320.2559                                          | Steroids               |
| 58   | 13.71       | (±)9-HpODE                                                  | [M-H] <sup>-</sup>                | C <sub>18</sub> H <sub>32</sub> O <sub>4</sub>                | -0.77       | 312.2298      | 311.2226                    | 311.2225, 293.2119, 211.1330, 171.1013            | Fatty acid derivatives |
| 59   | 14.38       | Phytosphingosine                                            | [M+H] <sup>+</sup>                | C <sub>18</sub> H <sub>39</sub> NO <sub>3</sub>               | 4.18        | 317.2930      | 318.3016                    | 60.0454, 318.3033, 282.2808, 300.2895             | Sphingolipids          |

Table 1 | Continued

| No. | $t_R$ (min) | Name                                             | Monitoring mode                     | Formula                                         | Error (ppm) | Calculated MW | Experimental mass (m/z) | MSMS data (+ or -) (m/z)                       | Classification         |
|-----|-------------|--------------------------------------------------|-------------------------------------|-------------------------------------------------|-------------|---------------|-------------------------|------------------------------------------------|------------------------|
| 60  | 14.38       | 2-Amino-1,3,4-octadecanetriol                    | [M+H] <sup>+</sup>                  | C <sub>18</sub> H <sub>39</sub> NO <sub>3</sub> | 4.48        | 317.2944      | 318.3017                | 318.3023, 300.2918, 282.2810                   | Sphingolipids          |
| 61  | 14.69       | Bavachinin                                       | [M+H] <sup>+</sup>                  | C <sub>21</sub> H <sub>22</sub> O <sub>4</sub>  | 5.06        | 338.1535      | 339.1608                | 339.1614, 147.0451, 219.1030, 69.0711          | Flavonoids             |
| 62  | 15.00       | 9S,13R-12-Oxophytodienoic acid                   | [M+H] <sup>+</sup>                  | C <sub>18</sub> H <sub>28</sub> O <sub>3</sub>  | 4.91        | 292.2053      | 293.2126                | 275.2026, 257.1917, 293.2133                   | Fatty acid derivatives |
| 63  | 15.06       | Sphingosine (d18:1)                              | [M+H-H <sub>2</sub> O] <sup>+</sup> | C <sub>18</sub> H <sub>33</sub> NO <sub>2</sub> | 4.87        | 299.2839      | 282.2806                | 282.2811, 252.2706, 264.2707, 95.0867, 56.0507 | Sphingolipids          |
| 64  | 15.28       | $\alpha$ -Linolenic acid                         | [M+H] <sup>+</sup>                  | C <sub>18</sub> H <sub>30</sub> O <sub>2</sub>  | 5.21        | 278.2260      | 279.2333                | 81.0711, 95.0867, 67.0554, 279.1337, 109.1023  | Fatty acid derivatives |
| 65  | 15.29       | ( $\pm$ )13-HODE                                 | [M-H] <sup>-</sup>                  | C <sub>18</sub> H <sub>32</sub> O <sub>3</sub>  | -2.24       | 296.2345      | 295.2272                | 295.2273, 195.1379, 277.2168, 113.0955         | Fatty acid derivatives |
| 66  | 15.59       | 20-Hydroxy-(5Z,8Z,11Z,14Z)-eicosatetraenoic acid | [M-H] <sup>-</sup>                  | C <sub>20</sub> H <sub>32</sub> O <sub>3</sub>  | -1.22       | 320.2348      | 319.2275                | 319.2274, 320.2313                             | Fatty acid derivatives |
| 67  | 15.68       | 9-Oxo-ODE                                        | [M+H] <sup>+</sup>                  | C <sub>18</sub> H <sub>30</sub> O <sub>3</sub>  | 4.08        | 294.2207      | 295.2280                | 277.2181, 179.1443                             | Fatty acid derivatives |
| 68  | 16.17       | 13(S)-HOTrE                                      | [M-H] <sup>-</sup>                  | C <sub>18</sub> H <sub>30</sub> O <sub>3</sub>  | -0.83       | 294.2193      | 293.2120                | 293.2118, 294.2152                             | Fatty acid derivatives |
| 69  | 16.19       | 9-Oxo-10(E),12(E)-octadecadienoic acid           | [M+H] <sup>+</sup>                  | C <sub>18</sub> H <sub>30</sub> O <sub>3</sub>  | 4.23        | 294.2207      | 295.2280                | 277.2181, 151.1129, 278.2214                   | Fatty acid derivatives |
| 70  | 17.66       | Oleamide                                         | [M+H] <sup>+</sup>                  | C <sub>18</sub> H <sub>35</sub> NO              | 4.89        | 281.2732      | 282.2805                | 282.2812, 265.2544, 247.2437, 83.0868          | Fatty acid derivatives |
| 71  | 18.16       | Palmitoyl ethanolamide                           | [M+H] <sup>+</sup>                  | C <sub>18</sub> H <sub>37</sub> NO <sub>2</sub> | 4.88        | 299.2839      | 300.2912                | 300.2918, 62.0613, 283.2649                    | Fatty acid derivatives |
| 72  | 18.57       | Oleoyl ethanolamide                              | [M+H] <sup>+</sup>                  | C <sub>20</sub> H <sub>39</sub> NO <sub>2</sub> | 5.96        | 325.2981      | 326.3073                | 326.3079, 62.0613                              | Fatty acid derivatives |
| 73  | 19.06       | Palmitoleic acid                                 | [M-H] <sup>-</sup>                  | C <sub>16</sub> H <sub>30</sub> O <sub>2</sub>  | -1.22       | 254.2243      | 253.2170                | 253.2168, 255.6895                             | Fatty acid derivatives |
| 74  | 19.27       | Betulin                                          | [M+H-H <sub>2</sub> O] <sup>+</sup> | C <sub>30</sub> H <sub>50</sub> O <sub>2</sub>  | 4.96        | 442.3833      | 425.3799                | 425.3832, 443.3915, 109.1023, 95.0867, 81.0711 | Triterpenoids          |
| 75  | 21.36       | Oleic acid                                       | [M-H] <sup>-</sup>                  | C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>  | -1.45       | 282.2555      | 281.2482                | 281.2482, 282.2516                             | Fatty acid derivatives |
| 76  | 21.54       | Anacardic acid                                   | [M-H] <sup>-</sup>                  | C <sub>22</sub> H <sub>36</sub> O <sub>3</sub>  | -1.35       | 348.2660      | 347.2587                | 303.2690, 347.2587                             | Phenolic acids         |
| 77  | 22.18       | Erucamide                                        | [M+H] <sup>+</sup>                  | C <sub>22</sub> H <sub>43</sub> NO              | 3.61        | 337.3357      | 338.3430                | 338.3443, 83.0867, 97.1024, 321.3176,          | Fatty acid derivatives |

## Research Article

Table 1 | Continued

| No. | $t_R$ (min) | Name                                 | Monitoring mode    | Formula                                           | Error (ppm) | Calculated MW | Experimental mass (m/z) | MSMS data (+ or -) (m/z)               | Classification         |
|-----|-------------|--------------------------------------|--------------------|---------------------------------------------------|-------------|---------------|-------------------------|----------------------------------------|------------------------|
| 78  | 23.41       | Erucic acid                          | [M-H] <sup>-</sup> | C <sub>22</sub> H <sub>42</sub> O <sub>2</sub>    | -1.58       | 338.3180      | 337.3107                | 337.3109, 338.3141                     | Fatty acid derivatives |
| 79  | 24.55       | Lupeol                               | [M+H] <sup>+</sup> | C <sub>30</sub> H <sub>50</sub> O                 | 4.49        | 426.3881      | 427.3954                | 427.3969, 95.0867, 109.1023            | Triterpenoids          |
| 80  | 24.70       | Phosphatidylinositol-1,2-dipalmitoyl | [M-H] <sup>-</sup> | C <sub>41</sub> H <sub>79</sub> O <sub>13</sub> P | -0.15       | 810.5257      | 809.5184                | 255.2325, 809.5182, 391.2245, 553.2767 | Phospholipids          |
| 81  | 24.72       | Linoleic acid                        | [M-H] <sup>-</sup> | C <sub>18</sub> H <sub>32</sub> O <sub>2</sub>    | -1.90       | 280.2397      | 279.2324                | 279.2326, 280.2363                     | Fatty acid derivatives |
| 82  | 25.81       | Cholest-4-en-3-one                   | [M+H] <sup>+</sup> | C <sub>27</sub> H <sub>44</sub> O                 | 4.93        | 384.3411      | 385.3484                | 385.3492, 109.0659, 97.0659            | Steroids               |

\*Compared with reference standards.

(CXCR2), transient receptor potential vanilloid 1 (TRPV1), hypoxia-inducible factor-1-alpha (HIF1A), and IL-6 (**Figure 3H**). CXCR2, HIF1A, TRPV1, and IL-6 demonstrated strong network interactions suggesting their central role in the therapeutic mechanisms of EH (**Figure 3I**). Enrichment analysis linked these targets to cellular responses to cytokine stimuli, acute inflammatory responses, and cytokine-mediated signaling pathways (**Figure 3J**). KEGG enrichment revealed shared pathways, including the IL-17, JAK-STAT, and estrogen signaling pathways, that were interconnected and critical in psoriasis (**Figure 3K**). IL-17 activates the JAK-STAT3 pathway and promotes Th17 differentiation, whereas estrogen inhibits this process, thereby balancing pro- and anti-inflammatory signaling. Additionally, CXCR2, HIF1A, TRPV1, and IL-6 have been implicated in psoriasis pathogenesis [34, 35]. CXCR2 promotes IL-17-induced neutrophil chemotaxis, HIF1A regulates glycolysis downstream of IL-17, and IL-6 amplifies IL-17 signaling, thereby driving chronic inflammation [3, 36]. TRPV1 stimulates IL-23 production through CGRP, and its inhibition decreases psoriatic lesions [37]. Given their close association with the IL-17 signaling pathway, further investigation of the active compounds of EH that target this key pathway is warranted.

### 3.5 Screening for bioactive compounds from EH with SPR-MS

The active compounds of EH were screened and identified with SPR-based ligand fishing followed by LC-MS analysis (**Figure 4A**). Given the central role of the IL-17 signaling pathway in psoriasis, we selected IL-6 and CXCL10, extracted by RNA-seq, as well as IL-17RA (a key receptor in the IL-17 signaling pathway) as baits for SPR-MS screening. Recombinant IL-17RA, IL-6, and CXCL10 proteins were immobilized on CM5 chips under optimal coupling pH conditions (**Supplementary Figure S5A-C**). The EH extracts exhibited specific binding to these proteins, whereas the negative control, rosiglitazone, showed no binding activity (**Supplementary Figure S5D-F**). EH binding to IL-17RA (**Figure 4B**), IL-6 (**Figure 4C**), and CXCL10 (**Figure 4D**) was concentration dependent, and 100 µg/mL was selected for SPR-MS analysis. The mass spectrometry data of EH recovered samples revealed that AL, methyl gallate (MG), and ellagic acid specifically bound IL-17RA (**Figure 4E**). Astragaloside (AS), rutin, and ellagic acid specifically bound IL-6 (**Figure 4F**). AL, ellagic acid, and apigenin specifically bound CXCL10 (**Figure 4G**). Isoquercitrin (IQ) exhibited broad binding affinity toward IL-17RA, IL-6, and CXCL10. The fragmentation patterns of the recovered samples aligned with those of the mixed standard (**Table 1**). Ellagic acid [38], rutin [39, 40], and apigenin [41, 42] (the aglycone form of apiin) have been reported to alleviate IMQ-induced psoriasis-like skin lesions. Therefore, our subsequent experiments focused on verifying the ability of AL, MG, AS, and IQ to alleviate psoriasis. The findings suggested that our strategy integrating network pharmacology and RNA-seq into SPR-MS is a promising approach for



## Research Article

(A) Venn diagram of EH targets and psoriasis targets. (B) PPI network of overlapping targets. (C, D) KEGG and GO enrichment of overlapping targets. (E) PPI network of the key functional module. (F, G) KEGG and GO enrichment of the key module. (H) Venn diagram of network pharmacology and RNA-seq targets. (I) PPI network of overlapping targets. (J) GO enrichment of overlapping targets. (K) Comparative analysis of KEGG enrichment results from network pharmacology and RNA-seq.

accurately screening active compounds from natural products.

### 3.6 Molecular docking analysis of active compounds and target proteins

The seven compounds included five flavonoids, which have similar structures, and two phenolic acids (**Supplementary Figure S6A**). Indeed, the activity of flavonoids, components known for their anti-inflammatory activity, is attributable to various pathways, including the modulation of proinflammatory cytokines, inactivation of transcription factors, and inhibition of regulatory enzymes that contribute to inflammation [43]. The interactions among the four candidate active compounds (AL, MG, AS, and IQ) and the proteins were predicted by molecular docking analysis. Protein-ligand interaction diagrams were obtained with LigPlot+, thus highlighting the optimal conformational hydrophobic bonds, hydrogen bonds, and bond lengths. Hydrogen bonds are indicated by green dashed lines, and hydrophobic interactions are depicted as red arcs (**Supplementary Figure S6B**). Detailed information regarding the binding interactions of the seven active compounds with the target proteins is provided in **Supplementary Figure S6C** and **Supplementary Table 1**. Notably, multiple hydrogen bonding interactions were observed between the active compounds and the surrounding amino acid residues of IL-17RA, IL-6, and CXCL10, thus indicating strong binding between the molecules. Additionally, flavonoid compounds had significantly higher binding energy scores toward the corresponding proteins than the other compounds, thus suggesting favorable potential for flavonoids in EH to treat psoriasis. The molecular docking results indicated that these compounds, particularly flavonoids, showed strong binding to these target proteins, thereby offering a promising foundation for further experimental validation and therapeutic development.

### 3.7 Validation of the ability of candidate compounds to alleviate skin lesions in mice

To further validate the accuracy of the SPR-MS strategy, we verified the efficacy of the screened AL, MG, AS, and IQ in treating psoriasis skin lesions in an IMQ-induced psoriasis mouse model (**Figure 5A**). IMQ-treated mice showed extensive erythema and scaling, which significantly decreased with AL, MG, AS, or IQ treatment; these compounds therefore mitigated psoriasis-like lesions (**Figure 5B**). Given AL's superior therapeutic efficacy to those of the other three components in treating psoriasis, we examined both high- and low-dose AL groups to investigate its potential dose-dependent

therapeutic effects. The PASI score also suggested that AL-H, AL-L, MG, AS, or IQ decreased the scaling score (**Figure 5C**), erythema score (**Figure 5D**), and total PASI score (**Figure 5E**). Compared with IMQ treatment, treatment with these active compounds effectively inhibited the IMQ-induced increase in ear thickness (**Figure 5F**). Moreover, the decrease in spleen index, by  $52.61\% \pm 11.33\%$ ,  $45.70\% \pm 16.97\%$ ,  $47.36\% \pm 14.29\%$ ,  $41.39\% \pm 16.16\%$ , and  $43.42\% \pm 10.50\%$  after AL-H, AL-L, MG, AS, and IQ interventions, respectively, suggested that the screened compounds attenuated the IMQ-induced immune response (**Figure 5G**). H&E staining revealed that AL-H, AL-L, MG, AS, and IQ ameliorated epidermal hyperplasia and hyperkeratosis (**Figure 5H**). The epidermal layer was decreased by  $62.72\% \pm 5.21\%$ ,  $56.18\% \pm 6.56\%$ ,  $47.68\% \pm 12.74\%$ ,  $55.72\% \pm 12.72\%$ , and  $50.96\% \pm 3.82\%$  after AL-H, AL-L, MG, AS, and IQ interventions, respectively (**Figure 5I**). These findings indicated that these compounds ameliorated IMQ-induced psoriasiform skin lesions. Among them, AL had the most pronounced therapeutic efficacy against psoriasis, with a distinct dose-dependent response.

Additionally, IF and IHC analyses revealed that the IMQ-induced increase in Ki67- and F4/80-positive cells significantly decreased after AL-H, MG, AS, and IQ intervention. The AL-H-treated mice exhibited the lowest epidermal thickness, Ki67 abundance, and F4/80 macrophage counts (**Figure 6A**). Western blotting was conducted to assess the effects of AL on the expression levels of key target proteins. The IMQ-induced model group had significantly upregulated protein expression of IL-17RA, IL-6, and CXCL10 (**Figure 6B**). In contrast, the AL-H-treated group exhibited marked downregulation of IL-17RA ( $48.00\% \pm 5.23\%$ ), IL-6 ( $41.40\% \pm 7.13\%$ ), and CXCL10 ( $57.23\% \pm 7.80\%$ ) protein expression in mouse skin tissue ( $p < 0.01$ , **Figure 6C**). The complete uncropped western blot images are presented in **Supplementary Figure 7A–C**. These findings suggested a strong correlation between AL's bioactivity and its modulatory effects on key targets, and further validated the rationale and precision of target selection for SPR-MS analysis. ELISA revealed elevated IL-6, CXCL10, IL-23, TNF- $\alpha$ , IL-17A, and IL-17F levels in IMQ-treated mice, which were markedly decreased by AL-H, AL-L, MG, AS, and IQ treatment (**Figure 6D–I**). Because AL-H showed superior efficacy to the other compounds in decreasing epidermal thickness, Ki67 abundance, F4/80 macrophage numbers, and inflammatory factor levels, we applied BLI analysis to confirm the direct interaction between AL and IL-17RA. The results revealed a direct and reversible interaction between AL and IL-17RA, with a dissociation constant ( $K_d$ ) of  $6.613 \mu\text{M}$  (**Figure 6J**). However, rosiglitazone,

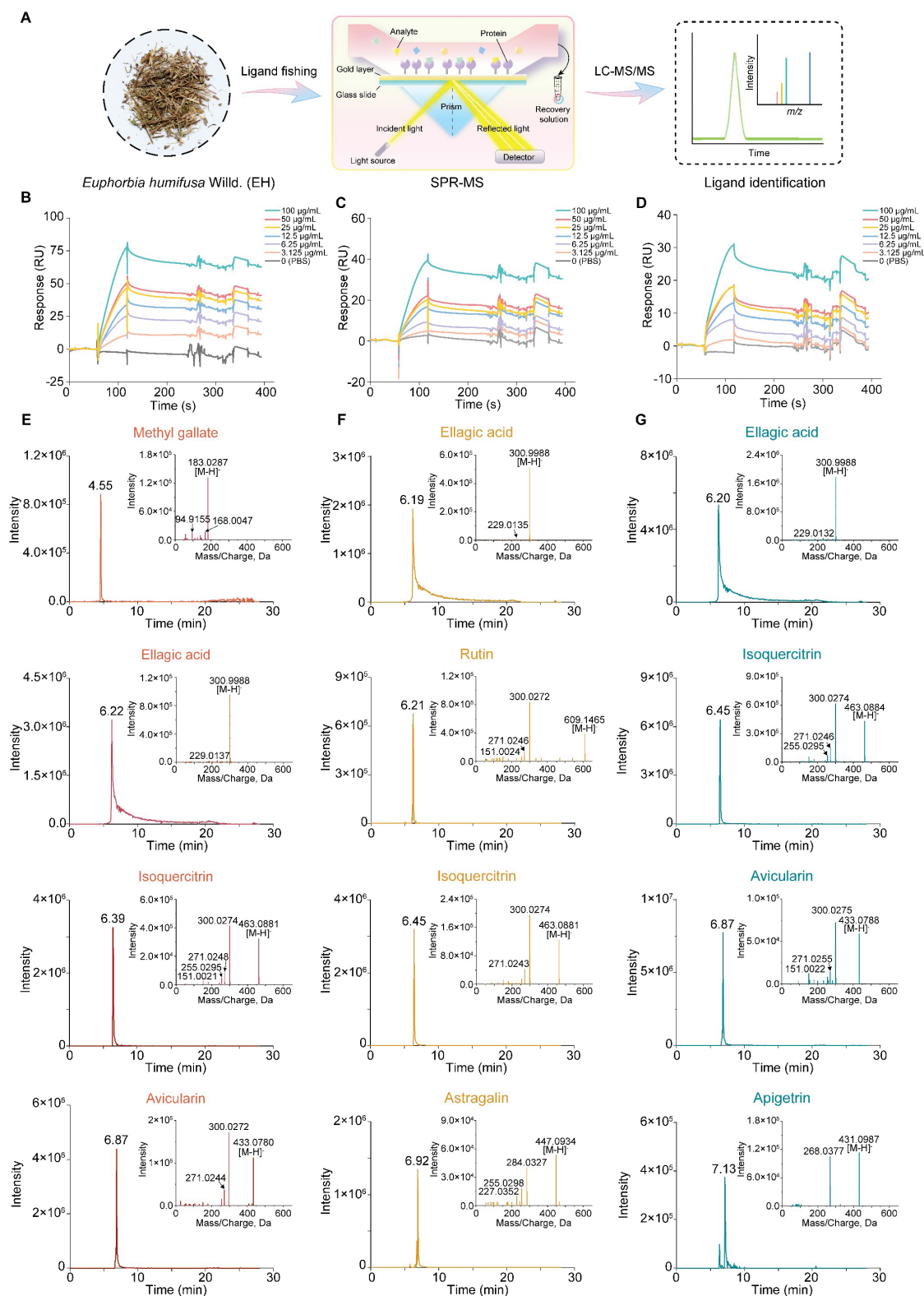


**Figure 4 | Recovery of target-bound compounds from EH.**

(A) Biacore fishing process. The prescreening response signals of EH extracts (0–100 µg/mL) on IL-17RA (B), IL-6 (C), and CXCL10 (D) are shown. Mass spectrograms of EH recovered samples for IL-17RA (E), IL-6 (F), and CXCL10 (G) are shown.

## Research Article

which was used as a negative control, did not bind IL-17RA (Figure 6K). Compared with brodalumab ( $K_d = 0.102$  nM), a positive control drug for IL-17RA, the affinity of AL for IL-17RA was high (Figure 6L). AL's slow binding response aligned with *in vivo* observations and suggested its potential as a lead compound for alleviating psoriasis. The SPR-MS strategy, combined with RNA-seq and network pharmacology, successfully identified AL, MG, AS, and IQ as psoriasis-alleviating compounds. Notably, AL had the most significant psoriasis-alleviating effect *in vivo*, in a dose-dependent manner.

### 3.8 Validation of the suitability and accuracy of the integrated screening strategy

AL, which showed optimal efficacy in treating psoriasis *in vivo*, was used to validate the accuracy of the integrated strategy for screening active compounds in complex natural products. IL-17RA was immobilized on a CM5 sensor chip, and AL and rosiglitazone (negative control) were injected into the SPR system (Supplementary Figure S8A). AL exhibited binding activity with IL-17RA, whereas rosiglitazone showed no binding, thus confirming the binding specificity of IL-17RA and AL (Supplementary Figure S8B). To further validate this strategy, we recovered AL (30  $\mu$ M) over 15 cycles on the SPR-MS system. The extracted ion chromatograms of the recovered AL sample and standard solution (10 mM) confirmed that AL was successfully screened and identified by the SPR-MS system (Supplementary Figure S8C,D). These findings validated the applicability and accuracy of the strategy combining network pharmacology and RNA-seq with SPR-MS for screening active compounds from EH, and highlighted its potential in elucidating the pharmacodynamic material basis of complex natural products.

Similarly to SPR biosensors, BLI is a powerful technique for characterizing the binding specificity, affinity, and kinetic dynamics of molecular interactions in real time [44]. When combined with LC-MS, BLI can capture and identify ligands bound to proteins immobilized on biosensor surfaces [45]. This integration offers an efficient high-throughput screening approach for studying active compounds in natural products. To enhance the suitability of our comprehensive strategy, we used SPR-MS to screen and BLI-MS to identify ligands in EH that bind IL-17RA (Supplementary Figure S9A). When BLI-MS was used with a high concentration of EH solution (1 mg/mL), this method identified three active compounds, MG, ellagic acid, and IQ, in agreement with the SPR-MS findings (Supplementary Figure S9B–D). However, with BLI-MS, in contrast to SPR-MS, the key active compound AL, which exhibits optimal activity for psoriasis treatment, was absent from the results. This discrepancy might be attributable to differences in sensitivity between the ligand fishing methods. Consequently, to ensure the suitability and accuracy of the screening strategy, we ultimately integrated the more sensitive

SPR-MS in the EH active compound screening with network pharmacology and RNA-seq.

## 4. DISCUSSION

IL-17 signaling is a well-established therapeutic axis in psoriasis, and the clinical success of IL-17RA antagonists such as brodalumab has confirmed the high value of this target [46]. Research on natural products for psoriasis has largely focused on broad anti-inflammatory effects, whereas studies precisely delineating their interaction with specific, critical nodes within this pathway, such as IL-17RA, have been lacking. This study addressed this gap by identifying AL as a novel IL-17 pathway inhibitor from the traditional Chinese medicine EH, through an integrated strategy using network pharmacology and RNA-seq (Figure 7). Although our results demonstrated that AL significantly downregulated the expression of IL-17RA and its key downstream effectors IL-6 and CXCL10, its precise mechanism of action is compellingly distinct from those of monoclonal antibodies. Whereas biologics block receptor-ligand interactions extracellularly, AL's intracellular multi-target suppression suggests that it might act through a different biochemical modality, potentially by modulating intracellular signal transduction [47, 48]. The primary focus of our study was to establish AL's efficacy and its central role in the anti-psoriatic activity of EH. In future studies, we plan to use techniques such as cellular thermal shift assays and drug affinity responsive target stability to biochemically validate whether AL directly engages IL-17RA or other components of its signaling complex. This approach will be critical to elucidating the exact protein target of AL and clarifying whether its effects are mediated through direct receptor antagonism or the regulation of downstream signaling events, thereby providing deeper mechanistic insights into its mode of action.

This study demonstrated that AL significantly suppresses the expression of IL-17RA, IL-6, and CXCL10. However, the underlying mechanism (i.e., whether it stems from specific inhibition of IL-17RA or results from broader anti-inflammatory effects) remains unclear, because of the lack of data on downstream signaling events such as NF- $\kappa$ B and STAT3. Accumulating evidence indicates that AL inhibits phosphorylation of I $\kappa$ B $\alpha$  and p65, thereby directly blocking NF- $\kappa$ B pathway activation, suppressing macrophage M1 polarization, and inhibiting NLRP3 inflammasome activation, which collectively contribute to its anti-inflammatory effects [14, 16]. Notably, AL's ability to curb cell proliferation in cutaneous squamous cell carcinoma suggests a potential role in directly modulating keratinocyte hyperproliferation in psoriasis via suppression of the MEK/NF- $\kappa$ B pathway [49]. Moreover, the observed decrease in IL-6 levels in this study suggested that AL might indirectly interfere with the IL-6–MEK/ERK/NF- $\kappa$ B–LMO4 axis, thereby ameliorating aberrant keratinocyte differentiation [50]. At a more upstream level, AL is likely to target TRAF6,

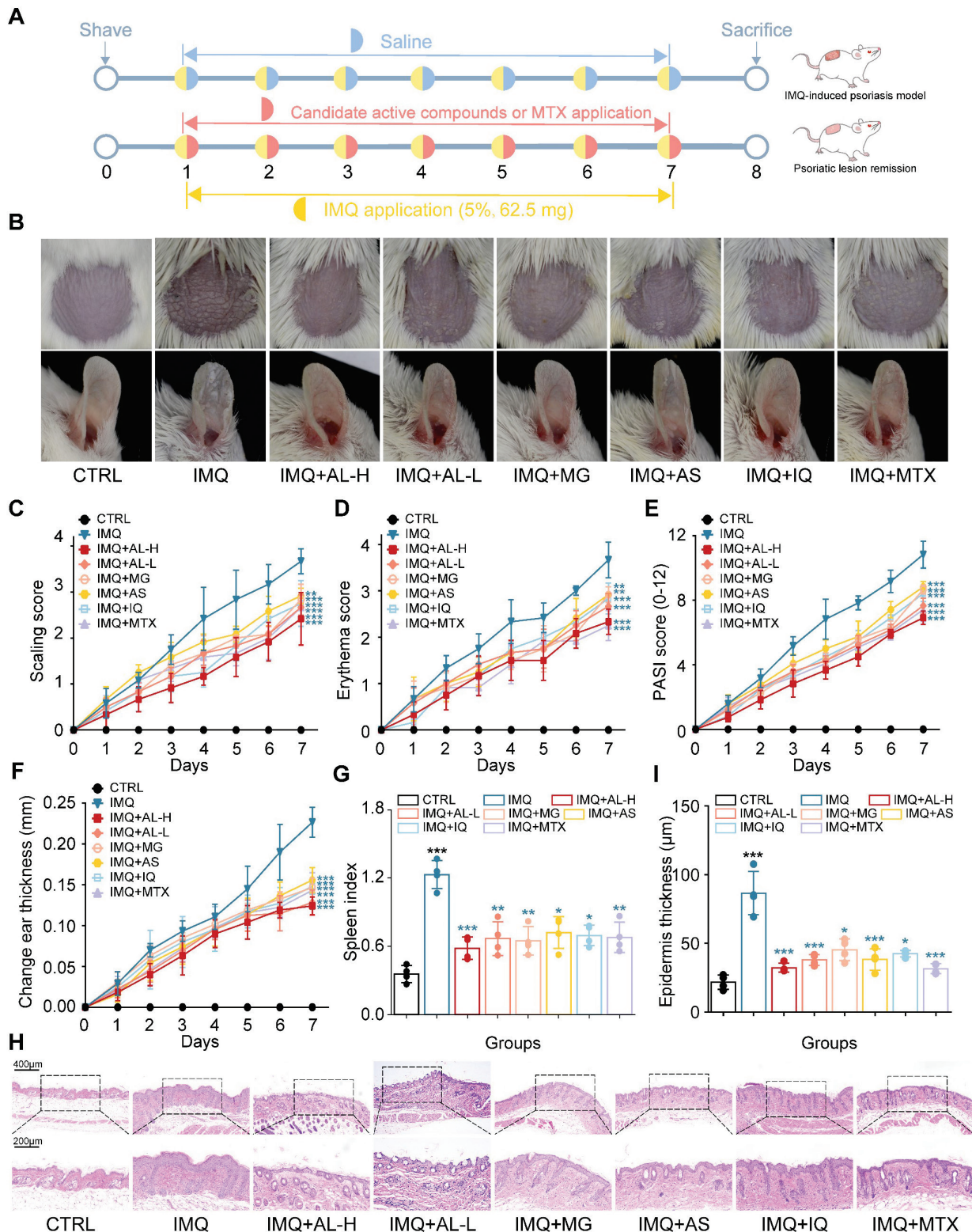


**Figure 5 | Candidate active compounds ameliorate IMQ-induced skin lesions.**

(A) Schemes of the IMQ-induced psoriasis-like model and active compound interventions. (B) Representative dorsal skin and ear images on day 8. (C–F) Scores for scaling, erythema, total PASI, and ear thickness changes.  $n = 6$ . Data are presented as mean  $\pm$  SD. (G) Spleen weight index.  $n = 4$ . Data are presented as mean  $\pm$  SD. (H, I) H&E staining and quantification of epidermal hyperplasia. Scale bar = 400  $\mu$ m and 200  $\mu$ m.  $n = 4$ . Data are presented as mean  $\pm$  SD.

## Research Article

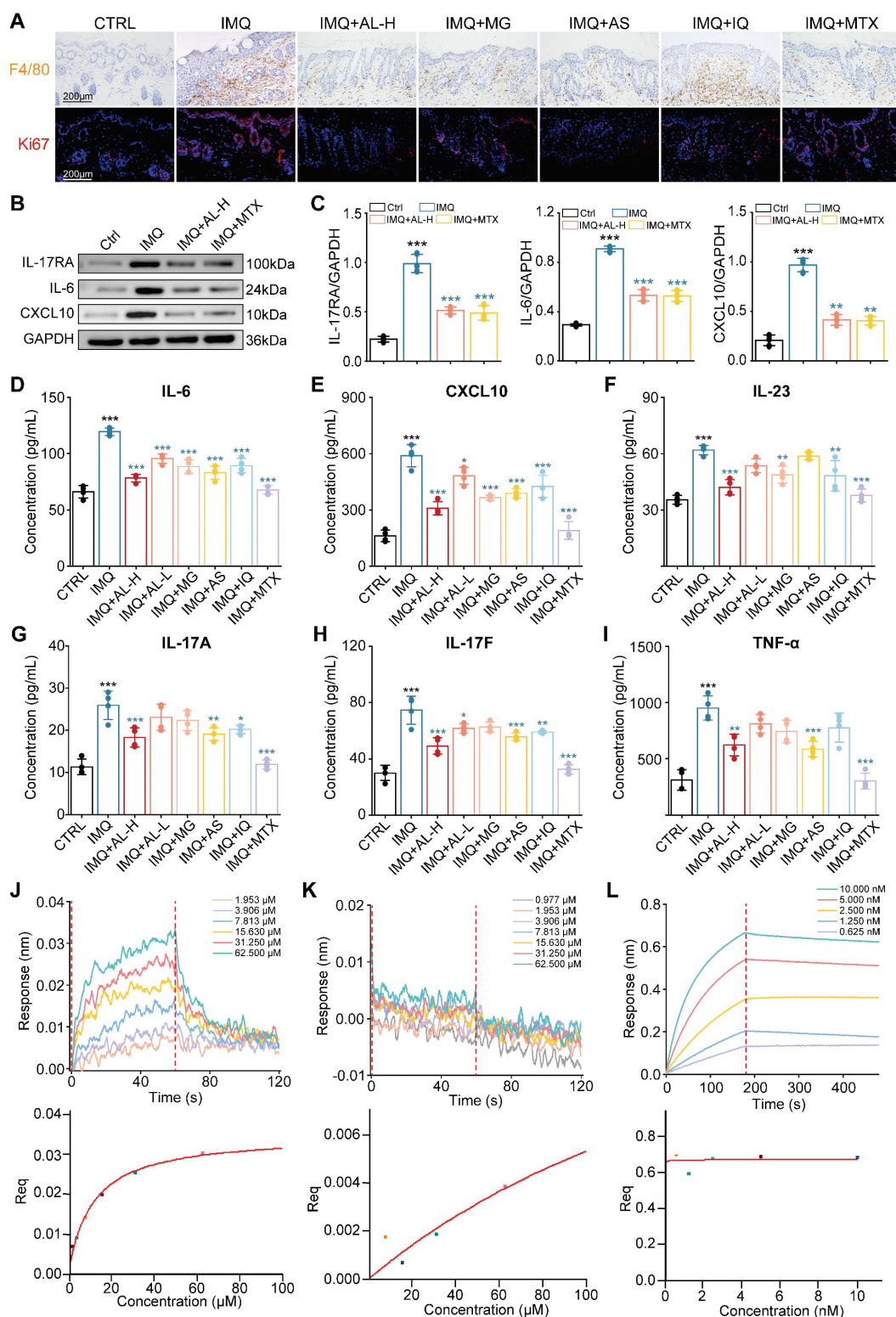


**Figure 6 | Candidate active compounds attenuate IMQ-induced epidermal hyperplasia and inflammation.**

(A) Immunohistochemistry and immunofluorescence staining of F4/80 or Ki67 in skin lesions on day 8 (scale bar, 200 μm). (B, C) Western blot analysis and quantification of key target proteins (IL-17RA, IL-6, and CXCL10) in skin tissue ( $n = 3$ ). (D–I) Serum levels of cytokines (IL-6, CXCL10, IL-23, IL-17A, IL-17F, and TNF-α), measured with ELISA ( $n = 4$ ). (J–L) Binding affinity of AL (J), the negative control drug rosiglitazone (K), and the positive control drug brodalumab (L) to IL-17RA, measured with BLI.



AL, because of its distinct advantages over both conventional small molecules and modern biologics, provides a compelling therapeutic candidate. Compared with representative small molecules such as quercetin and glycyrrhetic acid, AL offers superior drug-like properties and a targeted mechanism. Unlike the

broad-spectrum quercetin, which has poor solubility, AL's arabinose moiety enhances its aqueous solubility and facilitates specific hydrogen bonding interactions with IL-17RA/CXCL10, thus enabling more precise intervention targeting the IL-17 pathway [53-58]. Furthermore, although glycyrrhetic acid carries a risk of glucocorticoid-like adverse effects, AL's dual targeting of IL-17RA and CXCL10 does not pose such endocrine disruption concerns [59-61]. AL, compared with biologic IL-17RA inhibitors, has a micromolar affinity that is offset by its unique advantages as a small molecule: it allows for intracellular target engagement, convenient oral administration that enhances patient adherence, and a potentially diminished risk of immunosuppression through partial pathway modulation versus complete blockade [62]. Finally, its scalable synthesis from citrus byproducts presents a major economic advantage over costly biologic manufacturing [63, 64]. Collectively, this

## Research Article

unique combination of a targeted mechanism, favorable safety profile, oral availability, and cost-effectiveness establishes AL as a promising lead for next-generation psoriasis therapeutics.

Despite AL's therapeutic potential in alleviating psoriatic inflammation, as demonstrated by our mechanistic findings, its translational prospects are limited by considerable challenges. Like many flavonoids, AL is constrained by poor aqueous solubility, inadequate gastrointestinal absorption, and rapid metabolism, which collectively contribute to low oral bioavailability [65]. Although direct studies are lacking, structural analogy suggests its susceptibility to chemical instability and gut microbial modification, thus further compromising its delivery to target sites [66]. Moreover, its low natural abundance in sources such as EH and its complex purification requirements curtail final yield. Therefore, systematically evaluating its pharmacokinetics and toxicity, and developing strategies to overcome these limitations, will be critical next steps.

Integrated strategies involving structural modification, advanced drug delivery systems, and synthetic biology approaches may be systematically used to address these limitations. (1) Specific structural optimizations, such as incorporating substituted piperazine rings or developing pyrazole-flavone hybrids, have potential to strengthen anti-inflammatory activity through inhibition of key cytokines including IL-6 and TNF- $\alpha$ , as well as COX-2 enzyme activity [67]. Additionally, methylation and glycosylation modifications might improve metabolic stability and aqueous solubility, thereby optimizing its pharmacokinetic profile [68]. (2) Nanoformulation strategies offer another promising direction. Encapsulation of AL in functionalized nanocarriers has potential to significantly improve its solubility, skin permeation, and targeted delivery [62]. Such systems can enhance cutaneous retention and bioavailability, and potentially improve treatment outcomes and decrease systemic adverse effects [69]. (3) From a production perspective, synthetic biology platforms with engineered microbial hosts or cell-free systems enable high-yield production and precise enzymatic modification of flavonoid scaffolds [67, 70, 71]. (4) Innovative approaches such as proteolysis-targeting chimeras could be explored to conjugate AL with an E3 ligase ligand, to potentially enhance its selectivity and efficacy through targeted protein degradation [72]. Although these approaches provide a strong rationale for optimization, further experimental validation will be essential to confirm their feasibility for AL.

## 5. CONCLUSION

Herein, network pharmacology combined with RNA-seq was integrated with SPR-MS to elucidate the pharmacodynamic material basis and mechanisms of EH extracts for psoriasis alleviation. Seven active compounds were

screened from the 82 compounds in EH with SPR-MS, and four novel lead compounds, including AL, were found to alleviate psoriatic symptoms. *In vivo* experiments further confirmed that AL was the most effective compound in treating erythema and scaling symptoms in mice with IMQ-induced psoriasis. Although AL's broad anti-inflammatory properties were previously recognized, our work provides the first direct evidence, to our knowledge, of its specific binding to IL-17RA and its potent efficacy in alleviating psoriatic pathology *in vivo*. Mechanistic studies indicated that the significant inhibitory effects of EH extract and AL on inflammation and abnormal proliferation of keratinocytes in psoriasis might be associated with their modulatory effects on the IL-17 signaling pathway. These findings reposition AL from a general flavonoid to a targeted IL-17 pathway inhibitor, and offer a new strategic candidate for psoriasis therapy and a robust method for natural product research.

## DATA AVAILABILITY STATEMENT

The data from this study are available from the corresponding author upon reasonable request.

## ETHICS STATEMENT

Animals were treated and maintained in accordance with the Principles of Laboratory Animal Care, and all experiments were performed according to protocols approved by the Biology Ethics Committee of Shihezi University (ethical approval No. 2025-007, January 2, 2025).

## AUTHOR CONTRIBUTIONS

All authors contributed to study conception and design. Qian-Qian Zhou, Writing—original draft, Validation, Investigation, Formal analysis, Conceptualization. Zhe Wang, Writing—review & editing, Methodology. Yuan-Xiang Wang, Methodology, Investigation, Data curation. Jia-Wei Guan, Conceptualization. Lin-Yun He, Visualization. Ji-Wen Yang, Review, Editing. Ya-Ping Chi, Data curation. Li Zhao, Software, Investigation. Bo Zhang, Software, Investigation. Ke-Wu Zeng, Software, Investigation. Hua Zhang, Writing—review & editing, Conceptualization, Supervision. Bo Han, Project administration, Funding acquisition, Supervision. All authors have read and approved the final manuscript.

## ACKNOWLEDGEMENTS

This work was supported by the grants from the Financial science and technology plan project of Xinjiang (2023AB047, XJRC-2025BTKJ-PY-KJLJ-005, CZ000907); National Natural Science Foundation of China (82174075, 82574693); Jinan Science and Technology Achievements Transformation Promotion Project (202534027).

## CONFLICTS OF INTEREST

We have no known competing financial interests or personal relationships that could have appeared to influence the work reported herein.

## SUPPLEMENTARY MATERIAL

Supplementary Material can be downloaded from [https://amm-journal.org/wp-content/uploads/2026/03/amm20250058\\_Suppl.pdf](https://amm-journal.org/wp-content/uploads/2026/03/amm20250058_Suppl.pdf).

## REFERENCES

- [1] Gelfand JM: Psoriasis — More Progress but More Questions. *New England Journal of Medicine* 2024, 390:561–562.
- [2] Al-Horani R, Chui T, Hamad B: The Pipeline and Market for Psoriasis Drugs. *Nature Reviews Drug Discovery* 2024, 23:492–493.
- [3] Guo J, Zhang H, Lin W, Lu L, Su J, Chen X: Signaling Pathways and Targeted Therapies for Psoriasis. *Signal Transduction and Targeted Therapy* 2023, 8:1–38.
- [4] Sieminska I, Pieniąwska M, Grzywa TM: The Immunology of Psoriasis—Current Concepts in Pathogenesis. *Clinical Reviews in Allergy & Immunology* 2024, 66:164–191.
- [5] Ferrara F, Verduci C, Laconi E, Mangione A, Dondi C, Del Vecchio M, et al.: Therapeutic Advances in Psoriasis: From Biologics to Emerging Oral Small Molecules. *Antibodies* 2024, 13:76.
- [6] Koycheva IK, Marchev AS, Stoykova ID, Georgiev MI: Natural Alternatives Targeting Psoriasis Pathology and Key Signaling Pathways: A Focus on Phytochemicals. *Phytochemistry Reviews* 2023, 24:2147–2173.
- [7] Balato A, Burlando M, Campanati A, Costanzo A, Chiricozzi A, Gisondi P, et al.: Strategies for Optimal Use of Biological Therapies in Managing Psoriasis: Focus on Secukinumab. *Dermatology and Therapy* 2025, 15:3089–3108.
- [8] Konakanchi VC, Kar BR, Sathishkumar D, Tahiliani S, Parthasarathi A, Neema S, et al.: Small Molecules in the Management of Psoriasis and Psoriatic Arthritis. *Indian Journal of Dermatology* 2024, 69:249–255.
- [9] Xue T, Gong S, Xie TH, Li WJ, Huang JP: Development Status and Trend of Traditional Chinese Medicine Database. *TMR Modern Herbal Medicine* 2022, 5:19–26.
- [10] Wu X, Ma GL, Chen HW, Zhao ZY, Zhu ZP, Xiong J, et al.: Antibacterial and Antibiofilm Efficacy of the Preferred Fractions and Compounds from *Euphorbia Humifusa* (Herba Euphorbiae Humifusae) against *Staphylococcus Aureus*. *Journal of Ethnopharmacology* 2023, 306:116177.
- [11] Alimujiang A, Wang H, Chen L, Kang Y, Zhao J, Wei W, et al.: *Euphorbia Humifusa* Willd. Extract Alleviates Imiquimod-Induced Psoriasis-like Skin Lesions in Mice by Modulating the IL-17 Signaling Pathway. *Journal of Ethnopharmacology* 2025, 350:120030.
- [12] Huang PZ, Yang HY, Zhang LJ, Sun Y, Li Y, Gao K: Anti-Inflammatory Activities of Triterpenoids from *Euphorbia Humifusa* Willd. *Phytochemistry Letters* 2023, 54:129–132.
- [13] Lee SG, Kang H: In Vitro Adipocyte Differentiation Inhibition and In Vivo Effects on Lipid Metabolism in High-Fat Diet-Induced Obesity of *Euphorbia Humifusa*. *Journal of Microbiology and Biotechnology* 2024, 34:387–398.
- [14] Yang Y, Sheng D, Shi J, Xiao L, Wang Z, Yin Z, et al.: Avicularin Alleviates Osteoporosis-Induced Implant Loosening by Attenuating Macrophage M1 Polarization via Its Inhibitory Effect on the Activation of NF- $\kappa$ B. *Biomedicine & Pharmacotherapy* 2023, 158:114113.
- [15] Qiu T, Shi JX, Cheng C, Jiang H, Ruan HN, Li J, et al.: Avicularin Attenuates Lead-Induced Impairment of Hepatic Glucose Metabolism by Inhibiting the ER Stress-Mediated Inflammatory Pathway. *Nutrients* 2022, 14:4806.
- [16] Qiu T, Shi JX, Cheng C, Jiang H, Ruan HN, Li J, et al.: Hepatoprotective Effect of Avicularin on Lead-Induced Steatosis, Oxidative Stress, and Inflammation in Mice Associated with the MAPK/HSP60/NLRP3 and SREBP1c Pathway. *Toxicology Research* 2023, 12:417–424.
- [17] Lee WY, Park KI, Bak SB, Lee S, Bae SJ, Kim MJ, et al.: Evaluating Current Status of Network Pharmacology for Herbal Medicine Focusing on Identifying Mechanisms and Therapeutic Effects. *Journal of Advanced Research* 2024, 76:799–815.
- [18] Li L, Yang L, Yang L, He C, He Y, Chen L, et al.: Network Pharmacology: A Bright Guiding Light on the Way to Explore the Personalized Precise Medication of Traditional Chinese Medicine. *Chinese Medicine* 2023, 18:146.
- [19] Li X, Liu Z, Liao J, Chen Q, Lu X, Fan X: Network Pharmacology Approaches for Research of Traditional Chinese Medicines. *Chinese Journal of Natural Medicines* 2023, 21:323–332.
- [20] Wang Y, Xu T, Chen X, Ye Y, Liu L, Wang Y, et al.: Network Pharmacology and Molecular Docking Approach to Investigate the Mechanism of a Chinese Herbal Formulation Yougui Pills against Steroid-Related Osteonecrosis of the Femoral Head. *Arabian Journal of Chemistry* 2024, 17:105609.
- [21] Zhai Y, Liu L, Zhang F, Chen X, Wang H, Zhou J, et al.: Network Pharmacology: A Crucial Approach in Traditional Chinese Medicine Research. *Chinese Medicine* 2025, 20:8.
- [22] Li Q, Xiang Y, Zhang Z, Qu X, Wu J, Fu J, et al.: An Integrated RNA-Seq and Network Pharmacology Approach for Exploring the Preventive Effect of *Corydalis Bungeana* Turcz. Extract and Acetylcorynoline on LPS-Induced Acute Lung Injury. *Journal of Ethnopharmacology* 2024, 318:117048.
- [23] Wang C, Lin J, Xie H, Chen L, Chen P, Wu L, et al.: Study on Analgesic Effect of Shentong Zhuyu Decoction in Neuropathic Pain Rats by Network Pharmacology and RNA-Seq. *Journal of Ethnopharmacology* 2024, 330:118189.
- [24] Zhang P, Zhang D, Zhou W, Wang L, Wang B, Zhang T, et al.: Network Pharmacology: Towards the Artificial Intelligence-Based Precision Traditional Chinese Medicine. *Briefings in Bioinformatics* 2024, 25:bbad518.
- [25] Zhang Y, Wang D, Wang X, Ma H, Liu Y, Hong Z, et al.: A Dual-Target SPR Screening System for Simultaneous Ligand Discovery of SARS-CoV-2 Spike Protein and Its Receptor ACE2 from Chinese Herbs. *Journal of Pharmaceutical and Biomedical Analysis* 2024, 245:116142.
- [26] Xie J, Li XD, Li M, Zhu HY, Cao Y, Zhang J, et al.: Advances in Surface Plasmon Resonance for Analyzing Active Components in Traditional Chinese Medicine. *Journal of Pharmaceutical Analysis* 2024, 14:100983.
- [27] Deng R, Zong G, Wang X, Yue B, Cheng P, Tao R, et al.: Promises of Natural Products as Clinical Applications for Cancer. *Biochimica et Biophysica Acta (BBA) - Reviews on Cancer* 2025, 1880:189241.
- [28] Xun Y, Chen G, Tang G, Zhang C, Zhou S, Fong TL, et al.: Traditional Chinese Medicine and Natural Products in Management of Hepatocellular Carcinoma: Biological Mechanisms and Therapeutic Potential. *Pharmacological Research* 2025, 215:107733.
- [29] Zeng M, Feng A, Ren Y, Zhang Q, Wang R, Zhang B, et al.: *Salvia Miltiorrhiza* Bunge Extract and Przewalskin Ameliorate Bleomycin-Induced Pulmonary Fibrosis by Inhibition of Apoptosis, Oxidative Stress and Collagen

## Research Article

- Deposition via the TGF- $\beta$ 1 Pathway. *Phytomedicine* 2024, 125:155339.
- [30] Bharadwaj R, Lusi CF, Mashayekh S, Nagar A, Subbarao M, Kane GI, et al.: Methotrexate Suppresses Psoriatic Skin Inflammation by Inhibiting Muropeptide Transporter SLC46A2 Activity. *Immunity* 2023, 56:998–1012.
- [31] Tang D, Chen M, Huang X, Zhang G, Zeng L, Zhang G, et al.: SRplot: A Free Online Platform for Data Visualization and Graphing. *PLoS One* 2023, 18:e0294236.
- [32] Mastorino L, Dapavo P, Burzi L, Rosset F, Giunipero di Corteranzo I, Leo F, et al.: Drug Survival, Effectiveness, and Safety of Brodalumab for Moderate-to-Severe Psoriasis up to 3 Years. *Journal of the American Academy of Dermatology* 2024, 63:922–928.
- [33] Qiu YY, Zhang J, Zeng FY, Zhu YZ: Roles of the Peroxisome Proliferator-Activated Receptors (PPARs) in the Pathogenesis of Nonalcoholic Fatty Liver Disease (NAFLD). *Pharmacological Research* 2023, 192:106786.
- [34] Merrheim J, Villegas J, Van Wassenhove J, Khansa R, Berrih-Aknin S, le Panse R, et al.: Estrogen, Estrogen-like Molecules and Autoimmune Diseases. *Autoimmunity Reviews* 2020, 19:102468.
- [35] Li Y, Guo N, Zhao Y, Chen J, Zhao J, Bian J, et al.: IL-17A Activates JAK/STAT Signaling to Affect Drug Metabolizing Enzymes and Transporters in HepaRG Cells. *Molecular Immunology* 2024, 175:55–62.
- [36] Subudhi I, Konieczny P, Prystupa A, Castillo RL, Sze-Tu E, Xing Y, et al.: Metabolic Coordination between Skin Epithelium and Type 17 Immunity Sustains Chronic Skin Inflammation. *Immunity* 2024, 57:1665–1680.e7.
- [37] Chan TC, Lee MS, Huang WC, Chang WY, Krueger JG, Tsai TF: Capsaicin Attenuates Imiquimod-Induced Epidermal Hyperplasia and Cutaneous Inflammation in a Murine Model of Psoriasis. *Biomedicine & Pharmacotherapy* 2021, 141:111950.
- [38] Han YJ, Chung HS, Hwang HS: Ellagic Acid Suppresses  $\beta$ -Defensin2 Antimicrobial Peptide and CCL20 Chemokine in Psoriasis-like HaCaT Human Keratinocyte. *Advances in Traditional Medicine* 2025, 25:723–733.
- [39] Wang M, Ma X, Gao C, Luo Y, Fei X, Zheng Q, et al.: Rutin Attenuates Inflammation by Downregulating AGE-RAGE Signaling Pathway in Psoriasis: Network Pharmacology Analysis and Experimental Evidence. *International Immunopharmacology* 2023, 125:111033.
- [40] Wu P, Liu Y, Zhai H, Wu X, Liu A: Rutin Alleviates Psoriasis-Related Inflammation in Keratinocytes by Regulating the JAK2/STAT3 Signaling. *Skin Research and Technology* 2024, 30:e70011.
- [41] Meng X, Zheng S, Yin Z, Wang X, Yang D, Zou T, et al.: Apigenin Ameliorates Imiquimod-Induced Psoriasis in C57BL/6J Mice by Inactivating STAT3 and NF- $\kappa$ B. *Food Science and Human Wellness* 2024, 13:211–224.
- [42] Singh VK, Sahoo D, Aghahari K, Khan A, Mukhopadhyay P, Chanda D, et al.: Anti-Inflammatory, Anti-Proliferative and Anti-Psoriatic Potential of Apigenin in RAW 264.7 Cells, HaCaT Cells and Psoriasis like Dermatitis in BALB/c Mice. *Life Sciences* 2023, 328:121909.
- [43] Zawawi NA, Ahmad H, Madatheri R, Fadilah NIM, Maarof M, Fauzi MB: Flavonoids as Natural Anti-Inflammatory Agents in the Atopic Dermatitis Treatment. *Pharmaceutics* 2025, 17:261.
- [44] Jug A, Bratkovič T, Ilaš J: Biolayer Interferometry and Its Applications in Drug Discovery and Development. *TrAC Trends in Analytical Chemistry* 2024, 176:117741.
- [45] Guo M, Zhu F, Qiu W, Qiao G, Law BYK, Yu L, et al.: High-Throughput Screening for Amyloid- $\beta$  Binding Natural Small-Molecules Based on the Combinational Use of Biolayer Interferometry and UHPLC-DAD-Q/TOF-MS/MS. *Acta Pharmaceutica Sinica B* 2022, 12:1723–1739.
- [46] Babaei N, Gill M, Cervantes M, Kim D, Yang S, Wu JJ: Brodalumab for Moderate-to-Severe Psoriasis: A Comprehensive Review of Efficacy, Safety, and Clinical Positioning. *Biologics: Targets and Therapy* 2025, 19:415–422.
- [47] Gosia M, Doshi G, Bagwe Parab S, Godad A: Innovative Approaches to Psoriasis: Small Molecules Targeting Key Signaling Pathways. *Immunological Investigations* 2025, 54:457–493.
- [48] Azimi SI, Jafarzadeh A, Goodarzi A: Systematic Review of Comparative Studies on Emerging Psoriasis Treatments: Comparing Biologics with Biologics, Small Molecule Inhibitors with Small Molecule Inhibitors, and Biologics with Small Molecule Inhibitors. *Inflammopharmacology* 2025, 33:2377–2407.
- [49] Wang Y, Liu M, Chen S, Wu Q: Avicularin Inhibits Cell Proliferation and Induces Cell Apoptosis in Cutaneous Squamous Cell Carcinoma. *Experimental and Therapeutic Medicine* 2020, 19:1065–1071.
- [50] Tu Z, Wei W, Zeng F, Wang W, Zhang Y, Zhang Y, et al.: IL-6 Up-Regulates Expression of LIM-Domain Only Protein 4 in Psoriatic Keratinocytes through Activation of the MEK/ERK/NF- $\kappa$ B Pathway. *The American Journal of Pathology* 2024, 194:708–720.
- [51] Zou ZL, Sun MH, Yin WF, Yang L, Kong LY: Avicularin Suppresses Cartilage Extracellular Matrix Degradation and Inflammation via TRAF6/MAPK Activation. *Phytomedicine* 2021, 91:153657.
- [52] Zhou X, Xie J, Yu S, Li S, Chi C: Molecular and Functional Characterization of IL-17R Signaling Pathway-Related Genes in Cuttlefish *Sepiella Japonica*: Identification, Expression Pattern and Protein Interaction. *Aquaculture* 2026, 612:743295.
- [53] Kotik M, Kulik N, Valentová K: Flavonoids as Aglycones in Retaining Glycosidase-Catalyzed Reactions: Prospects for Green Chemistry. *Journal of Agricultural and Food Chemistry* 2023, 71:14890–14910.
- [54] Xie L, Deng Z, Zhang J, Dong H, Wang W, Xing B, et al.: Comparison of Flavonoid O-Glycoside, C-Glycoside and Their Aglycones on Antioxidant Capacity and Metabolism during In Vitro Digestion and In Vivo. *Foods* 2022, 11:882.
- [55] Ali AU, Khallaf ISA, Kamel AA, Badran AY, Gomaa AS, El faham TH, et al.: Impact of Quercetin Spanlastics on Livin and Caspase-9 Expression in the Treatment of Psoriasis Vulgaris. *Journal of Drug Delivery Science and Technology* 2022, 76:103809.
- [56] Zhang Y, Gong S, Liu L, Shen H, Liu E, Pan L, et al.: Cyclodextrin-Coordinated Liposome-in-Gel for Transcutaneous Quercetin Delivery for Psoriasis Treatment. *ACS Applied Materials & Interfaces* 2023, 15:40228–40240.
- [57] Liu Q, Liu J, Zheng Y, Lei J, Huang J, Liu S, et al.: Quercetin Mediates the Therapeutic Effect of Centella Asiatica on Psoriasis by Regulating STAT3 Phosphorylation to Inhibit the IL-23/IL-17A Axis. *Nan Fang Yi Ke Da Xue Xue Bao* 2025, 45:90–99.
- [58] Ma J, Wang F, Wang L, Wang Y, Wu D, Jiang W, et al.: Inhibition of PI3K/AKT/GLUT1 Signaling Pathway by Quercetin in the Treatment of Psoriasis. *Frontiers in Bioscience-Landmark* 2025, 30:26884.

- [59] Sabbadin C, Graziani A, Bavaresco A, Mazzeo P, Tizianel I, Ceccato F, et al.: Pseudohyperaldosteronism Due to Licorice: A Practice-Based Learning from a Case Series. *International Journal of Molecular Sciences* 2024, 25:7454.
- [60] Li WX, Lu YF, Wang F, Ai B, Jin SB, Li S, et al.: Application of 18 $\beta$ -Glycyrrhetic Acid in the Structural Modification of Natural Products: A Review. *Molecular Diversity* 2025, 29:739–781.
- [61] Wei J, Zhang J, Hu F, Zhang W, Wu Y, Liu B, et al.: Anti-Psoriasis Effect of 18 $\beta$ -Glycyrrhetic Acid by Breaking CCL20/CCR6 Axis through Its Vital Active Group Targeting GUSB/ATF2 Signaling. *Phytomedicine* 2024, 128:155524.
- [62] Kadagothy H, Nene S, Amulya E, Vambhurkar G, Rajalakshmi AN, Khatri DK, et al.: Perspective Insights of Small Molecules, Phytoconstituents and Biologics in the Management of Psoriasis: A Focus on Targeting Major Inflammatory Cytokine Pathways. *European Journal of Pharmacology* 2023, 947:175668.
- [63] Figueira O, Pereira V, Castilho PC: A Two-Step Approach to Orange Peel Waste Valorization: Consecutive Extraction of Pectin and Hesperidin. *Foods* 2023, 12:3834.
- [64] Ji Z, Deng W, Chen D, Liu Z, Shen Y, Dai J, et al.: Recent Understanding of the Mechanisms of the Biological Activities of Hesperidin and Hesperetin and Their Therapeutic Effects on Diseases. *Heliyon* 2024, 10:e26862.
- [65] Kumar RM, Kumar H, Bhatt T, Jain R, Panchal K, Chaurasiya A, et al.: Fisetin in Cancer: Attributes, Developmental Aspects, and Nanotherapeutics. *Pharmaceuticals* 2023, 16:196.
- [66] Pérez-Valero Á, Magadán-Corpas P, Ye S, Serna-Diestro J, Sordon S, Hus E, et al.: Antitumor Effect and Gut Microbiota Modulation by Quercetin, Luteolin, and Xanthohumol in a Rat Model for Colorectal Cancer Prevention. *Nutrients* 2024, 16:1161.
- [67] Sajid M, Channakesavula CN, Stone SR, Kaur P: Synthetic Biology towards Improved Flavonoid Pharmacokinetics. *Biomolecules* 2021, 11:754.
- [68] Hu L, Luo Y, Yang J, Cheng C: Botanical Flavonoids: Efficacy, Absorption, Metabolism and Advanced Pharmaceutical Technology for Improving Bioavailability. *Molecules* 2025, 30:1184.
- [69] Li Y, Cheng Y, Cai Y, Duan Z, Xu H, Huang Y, et al.: Novel Small-Molecule Treatment and Emerging Biological Therapy for Psoriasis. *Biomedicines* 2025, 13:781.
- [70] Shen T, Wang X, Zhang X, Pei J, Wang Z, Li Q, et al.: Engineering of Flavonoid 3'-O-Methyltransferase for Improved Biomodification of Fisetin in *Escherichia Coli*. *Journal of Agricultural and Food Chemistry* 2025, 73:11132–11145.
- [71] Wang J, Chen C, Guo Q, Gu Y, Shi TQ: Advances in Flavonoid and Derivative Biosynthesis: Systematic Strategies for the Construction of Yeast Cell Factories. *ACS Synthetic Biology* 2024, 13:2667–2683.
- [72] Cai J, Chen C, Wang J, Zhang X, Cui Y, Zhu Q, et al.: PROTAC: A Revolutionary Technology Propelling Small Molecule Drugs into the next Golden Age. *Frontiers in Oncology* 2025, 15:1676414.