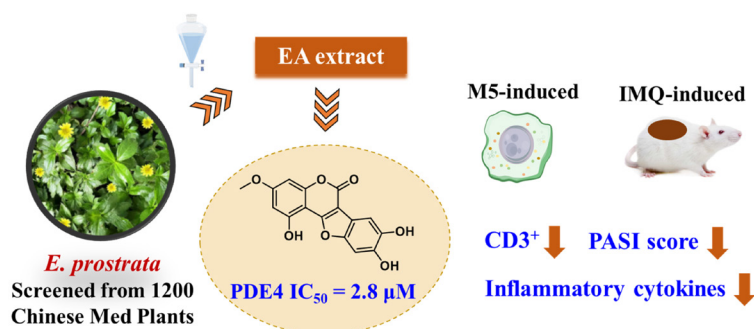


Discovery of wedelolactone from *Eclipta Prostrata* (L.) Linn. as a natural PDE4 inhibitor with potent anti-psoriasis effects

Graphical abstract



Highlights

- Wedelolactone (WDL), identified through bioactivity-guided isolation from *Eclipta prostrata* (L.) Linn., potently inhibits PDE4 ($IC_{50} = 2.8 \mu M$) and stabilizes binding as revealed by molecular dynamics simulations.
- WDL exerted potent anti-psoriatic effects both *in vitro* and *in vivo* by suppressing pro-inflammatory cytokines and restoring cAMP levels, showing superior efficacy to calcipotriol.
- WDL demonstrates a favorable safety profile and metabolic stability, supporting its potential as a safe topical agent for long-term management of psoriasis.

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In brief

Wedelolactone, a natural product isolated from *Eclipta prostrata* (L.) Linn., was identified as a potent PDE4 inhibitor that suppresses psoriasis-associated inflammation and epidermal hyperplasia. With superior efficacy to calcipotriol, favorable metabolic stability, and good topical safety, it holds strong promise as a PDE4-targeting therapy for psoriasis.

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Discovery of wedelolactone from *Eclipta Prostrata* (L.) Linn. as a natural PDE4 inhibitor with potent anti-psoriasis effects

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ABSTRACT

Psoriasis is a chronic inflammatory disorder for which phosphodiesterase-4 (PDE4) inhibitors have emerged as promising therapeutic agents due to an ability to regulate inflammatory signaling pathways. In this study 1200 methanolic extracts from Chinese medicinal plants were screened and *Eclipta prostrata* (L.) Linn. (*E. prostrata*) was shown to have potent PDE4 inhibitory activity. Bioassay-guided fractionation further demonstrated that the ethyl acetate (EA) fraction exhibited the highest inhibitory activity, leading to the isolation of wedelolactone (WDL) as the principal bioactive compound ($IC_{50} = 2.8 \mu M$). Molecular dynamics simulations revealed that WDL forms stable interactions with PDE4D through hydrogen bonding and hydrophobic contacts. *E. prostrata*-EA and WDL significantly suppressed pro-inflammatory cytokines in keratinocytes *in vitro*. Topical application of WDL demonstrated superior anti-psoriatic efficacy compared to calcipotriol *in vivo*, as evidenced by reduced Psoriasis Area and Severity Index scores, normalized epidermal thickness, and improved inflammatory cytokine profiles. WDL exhibited favorable metabolic stability in liver microsomes and demonstrated a good safety profile in subacute toxicity assessments with no systemic toxicity. These findings established WDL as a potent and safe topical PDE4 inhibitor, highlighting the potential of WDL as a novel therapeutic candidate for psoriasis and warranting further clinical development.

Keywords: *Eclipta prostrata* (L.) Linn., Wedelolactone, Phosphodiesterase-4 inhibitor, Psoriasis, Molecular dynamics simulation

1. INTRODUCTION

Psoriasis is a chronic, immune-mediated inflammatory skin disease characterized by erythematous plaques covered with silvery-white scales. Patients often have symptoms, such as itching, burning, dryness, cracking, and in severe cases, bleeding [1, 2]. The global prevalence of psoriasis is on the rise and poses a significant burden on patients and healthcare systems. Epidemiologic studies conducted in China have reported a psoriasis prevalence of approximately 0.47%, affecting >7 million individuals [3]. Despite the availability of various treatments, managing psoriasis effectively remains challenging due to limitations in efficacy, safety, and patient adherence.

Topical therapies, such as corticosteroids and vitamin D analogues (e.g., calcipotriol), are commonly used but often provide only temporary relief and may cause side effects, like skin atrophy and irritation, with long-term use [4, 5]. Systemic agents, such as methotrexate and cyclosporine, offer more potent anti-psoriatic effects but are associated with hepatotoxicity, nephrotoxicity, myelosuppression, and an increased risk of infections, which require close laboratory and clinical monitoring [6, 7]. Biologic therapies targeting immune mediators, including tumor necrosis factor- α (TNF- α), interleukin (IL)-17 A, and IL-23 inhibitors, have revolutionized psoriasis treatment by offering improved efficacy and specificity [8-10]. However, the high cost, risk of

immunosuppression-related infections, and inconvenience of parenteral administration limit widespread use [11, 12]. Given these challenges, there is a growing interest in developing alternative therapies that are effective, safe for long-term use, and more accessible to patients.

Herbal medicines have attracted attention due to multi-targeted mechanisms of action and lower incidence of adverse effects [13–15]. Medicinal plants, which have traditionally been used for skin diseases, may provide anti-inflammatory, immunomodulatory, and antiproliferative effects that are beneficial in psoriasis management [16, 17].

Phosphodiesterase-4 (PDE4) is a key enzyme responsible for hydrolysis of cyclic adenosine monophosphate (cAMP) [18, 19]. By modulating cAMP levels, PDE4 regulates inflammatory responses, thereby influencing the production of pro-inflammatory and anti-inflammatory mediators, and making PDE4 an effective target for psoriasis treatment [20, 21]. Oral PDE4 inhibitors, such as apremilast, have been approved for the treatment of moderate-to-severe plaque psoriasis, demonstrating the therapeutic potential of targeting PDE4 [22]. However, use of apremilast is associated with several adverse effects, including gastrointestinal symptoms (nausea and diarrhea), headaches, weight loss, and in some cases, depression and mood changes.

Recently, a PDE4 inhibitor screening campaign was launched using our in-house Chinese medicinal plant extract library, which is comprised of 1200 plants. Among these extracts, the methanolic extract of *Eclipta prostrata* (L.) Linn (*E. prostrata*) demonstrated notable PDE4 inhibitory activity, achieving 95.2% and 56.6% inhibition at 10 and 1 $\mu\text{g/mL}$, respectively (Figure 1A and Table 2). *E. prostrata*, also known as *Eclipta alba* (L.) Hassk. and commonly referred to as false daisy, is a member of the Asteraceae family [23]. This herbaceous plant thrives in damp environments and contains abundant bioactive substances, including phenolic compounds, alkaloids, triterpenes, and steroids [24–26]. To further explore the active components of *E. prostrata* responsible for PDE4 inhibition, bioassay-guided fractionation and characterization were performed and the potential anti-psoriasis effects were assessed using *in vitro* and *in vivo* models.

2. METHODS

2.1 Plant extract preparation

A comprehensive library of extracts from 1200 Chinese medicinal plants was established. Each plant was systematically classified, cleaned, air-dried, and pulverized to obtain fine dry powders. Approximately 2–5 kg of each plant was macerated with 3 $\times$ 10 L of methanol (MeOH) for 24 h, followed by filtration. The combined filtrates were concentrated under reduced pressure to yield methanolic (crude) extracts. Each methanolic extract was then suspended in 3 L of water (H_2O) and sequentially partitioned with 3 $\times$ 3 L of petroleum ether (PE) and 3 $\times$ 3 L of ethyl acetate (EA). The organic phases and

the aqueous phase were concentrated under reduced pressure to obtain the corresponding PE, EA, and H_2O fractions (Figure 1A).

2.2 *In vitro* enzymatic activity assay

Recombinant human PDE4D2 (amino acids 86–413) was expressed in *Escherichia coli* BL21 (CodonPlus) cells transformed with the pET15b-PDE4D2 plasmid. Cultures were grown at 37 $^{\circ}\text{C}$ in Luria-Bertani (LB) medium to an OD600 of 0.6, then induced with 0.1 mmol/L isopropyl- β -D-thiogalactopyranoside (IPTG) at 16 $^{\circ}\text{C}$ for 24 h. Cells were harvested and lysed, and the supernatant was purified using Ni-NTA affinity chromatography, ion exchange, and size-exclusion chromatography, yielding PDE4D2 protein with a purity >95%.

The PDE4 enzymatic activity was measured using a radioisotope assay. The reaction mixture contained 20 mmol/L Tris-HCl (pH 7.5), 10 mmol/L MnCl_2 , 1 mmol/L dithiothreitol (DTT), and 20,000–30,000 cpm of ^3H -cAMP substrate in a final volume of 100 μL . Purified PDE4D2 (final concentration, 2 nM) and test compounds at various concentrations were added to the reaction mixture. After incubation at 25 $^{\circ}\text{C}$ for 15 min, the reaction was terminated by adding 200 μL of 0.2 mol/L ZnSO_4 and 200 μL of 0.2 mol/L $\text{Ba}(\text{OH})_2$. The mixture was centrifuged and the supernatant containing unreacted ^3H -cAMP was transferred to 2.5 mL of scintillation cocktail. Radioactivity was measured using a PerkinElmer MicroBeta liquid scintillation counter (Waltham, MA, USA). IC_{50} values were determined by testing at least eight concentrations of each compound with each measurement performed in triplicate.

2.3 Purification of compounds from *E. prostrata*-EA

One gram of *E. prostrata*-EA was subjected to reverse-phase C18 gel column chromatography and eluted with a MeOH/ H_2O gradient (10%–100% MeOH in H_2O) at a flow rate of 10 mL/min over a total of 500 mL to afford 2 primary fractions, which were further purified using semi-preparative high-performance liquid chromatography [pre-HPLC] (Agilent 1260 Infinity II, YMC-Pack Pro C18 RS column; Santa Clara, CA, USA). Elution was performed with an acetonitrile (CH_3CN)/ H_2O gradient (8%–90% CH_3CN in H_2O) at a flow rate of 3 mL/min over 40 min, yielding compound 1 (15 mg; t_R , 14.980 min) and compound 2 (180 mg; t_R , 17.908 min; Figure 1).

2.4 Structural characterization of compounds 1 and 2

Compounds 1 and 2 were characterized using nuclear magnetic resonance (NMR) spectroscopy and high-resolution mass spectrometry (HRMS). ^1H NMR spectra were performed on a Bruker AVANCE III HD 400 MHz digital NMR spectrometer (Billerica, MA, USA). The samples were prepared in deuterated solvent ($\text{DMSO}-d_6$). HRMS was performed using an Agilent 1290 infinity II (Santa

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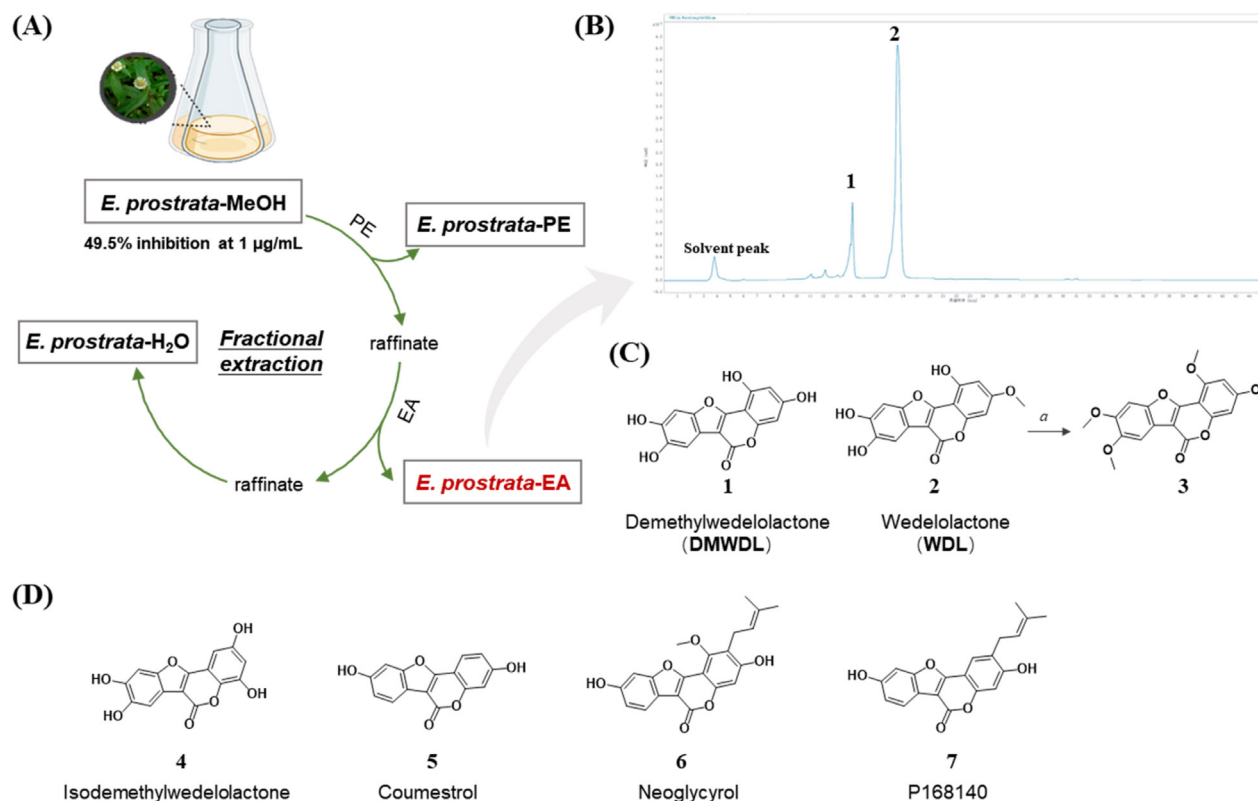


Figure 1 | Fractionation, purification, and structural characterization of *E. prostrata*. (A) Partitioning of *E. prostrata* leading to fractions with different polarities. (B) HPLC chromatogram presented only two principal components in *E. prostrata*-EA. (C) Chemical structures of compounds 1–3; reagents and conditions of *a*: dimethyl sulfate; K₂CO₃; acetone; 0 °C-rt; 6 h; and yield, 55%. (D) Chemical structures of compounds 4–7.

Table 1 | Primer sequences used for qRT-PCR analysis in M5-induced HaCaT cells.

Genes	Forward primer	Reverse primer
β-actin	CCAACCGCGAGAAGATGA	CCAGAGGCGTACAGGGATAG
IL-1α	AGATGCCTGAGATACCCAAAACC	CCAAGCACACCCAGTAGTCT
IL-1β	ATGATGGCTTATTACAGTGGCAA	GTCGGAGATTCGTAGCTGGA
IL-8	AAGGAAAACCTGGGTGCAGAG	ATTGCATCTGGCAACCCTAC
CXCL-1	AGGGAATTCACCCCAAGAAC	TGGATTGTCACTGTTTCAGCA
CCL-2	AAGACCATTGTGGCCAAGGA	TTCGGAGTTTGGGTTTGCT

Clara, CA, USA) and Waters UPLC G2-XS mass spectrometer (Milford, MA, USA) equipped with an electrospray ionization (ESI) source (Waters, Milford, MA, USA).

2.5 Synthesis and procurement of compounds 3–7

The methylated derivative (compound 3) of compound 2 was synthesized as follows. Compound 2 (100 mg) was dissolved in acetone (5 mL) and cooled to 0 °C. Potassium carbonate (200 mg) and dimethyl sulfate (0.1 mL) were added dropwise with constant stirring. The reaction

mixture was gradually warmed to room temperature and stirred for 6 h. Upon completion, which was monitored by thin layer chromatography, the reaction was quenched with H₂O, extracted with EA, dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The crude product was purified by column chromatography to yield compound 3 (55 mg; 55% yield). Additionally, four structurally similar compounds (compounds 4–7) were obtained from Innochem Co., Ltd. (Beijing, China).

Table 2 | Inhibition activity of the *E. prostrata* extracts and compounds against PDE4D.

Extracts/Compounds	PDE4D Inhibitory Rate (%)		IC ₅₀ (μM)
	10 μg/mL	1 μg/mL	
<i>E. prostrata</i> -MeOH	95.2	49.5	<i>n.s.</i> ^b
<i>E. prostrata</i> -PE	81.7	33.5	<i>n.s.</i> ^b
<i>E. prostrata</i> -EA	100.0	56.6	<i>n.s.</i> ^b
<i>E. prostrata</i> -H ₂ O	42.6	<i>n.s.</i> ^b	<i>n.s.</i> ^b
DMWDL (1)	100.0	86.7	3.5 ± 0.4
WDL (2)	100.0	91.1	2.8 ± 0.1
3	71.6	10.8	<i>n.s.</i> ^b
4	71.3	30.2	<i>n.s.</i> ^b
5	90.9	54.7	<i>n.s.</i> ^b
6	66.7	38.1	<i>n.s.</i> ^b
7	90.2	26.5	<i>n.s.</i> ^b
Rolipram ^a	56.2	<i>n.s.</i> ^b	0.59 ± 0.05

^aPositive control, at a concentration 0.8 μM. ^bActivity was not tested.

2.6 Molecular dynamics (MD) simulation

Molecular docking was performed using the CDOCKER program and implemented in the Accelrys Discovery Studio (San Diego, CA, USA). The co-crystal structure of PDE4D-roflumilast (PDB code, 1XOQ) was utilized and docking parameters were similar to the previously reported docking parameters [27, 28]. MD simulations were subsequently performed using GROMACS 2022 (<https://manual.gromacs.org/2022/>). The General Amber Force Field (GAFF) was applied for the ligand and the AMBER14SB force field was used for the protein. The complex was placed in a cubic box with a 10 Å buffer of TIP3P water molecules. Sodium ions were added to neutralize the system. Energy minimization was performed to remove bad contacts, followed by equilibration under NVT and NPT ensembles for 100 ps each. The production MD simulation was run for 100 ns at 298 K and 1 bar with periodic boundary conditions. The Particle Mesh Ewald method [29] was used for long-range electrostatics. Trajectories were saved every 10 ps for analysis. The MM/PBSA method was used to calculate binding free energies using the g_mmpbsa tool.

2.7 Cell viability assay on HaCaT cells

HaCaT cells were cultured in Dulbecco's modified Eagle medium [DMEM] (Hyclone, Logan, Utah, USA) supplemented with 10% fetal bovine serum (BioExplorer, San Diego, CA, USA) and 1% penicillin-streptomycin-gentamicin solution (Servicebio, Wuhan, Hubei, China) at 37 °C in a 5% CO₂ atmosphere. Cells were seeded on 96-well plates at a density of 5 × 10³ cells/well and allowed

to attach overnight. The cells were then treated with various concentrations of *E. prostrata*-EA (1.5650–400 μg/mL) and WDL (1.5650–400 μM) for 72 h. Cell viability was assessed using the Cell Counting Kit-8 (CCK-8) assay (TargetMol, Shanghai, China) according to the manufacturer's instructions. Absorbance was measured at 450 nm using a microplate reader (Agilent BioTek SYNERGY H1).

2.8 In vitro M5-induced HaCaT cell assay

HaCaT cells were serum-starved for 24 h, then stimulated with a combination of 5 cytokines (M5) to simulate the inflammatory state of psoriatic keratinocytes: interleukin (IL)-17A (10 ng/mL); IL-22 (10 ng/mL); IL-1α (10 ng/mL); tumor necrosis factor-α [TNF-α] (10 ng/mL); and oncostatin M [OSM] (10 ng/mL). The cells were treated with *E. prostrata*-EA (100 μg/mL) or WDL (100 μM) for 24 h while induced with the M5. The levels of inflammatory cytokine (IL-1α, IL-1β, and IL-8) and chemokine (CXCL-1 and CCL-2) expression were measured using quantitative real-time PCR [qRT-PCR] (Roche 480, Mannheim, Baden-Württemberg, Germany; Table 1).

2.9 Preparation of ointments

Topical ointments containing *E. prostrata*-EA or WDL were formulated using an emulsification process. The oil phase consisted of stearic alcohol (1.8 g), white petrolatum (2.0 g), liquid paraffin (1.3 mL), and the active ingredient (*E. prostrata*-EA at 10% w/w; WDL at 2%, 5%, or 15% w/w). The mixture was heated to 80 °C with constant stirring. In parallel, the aqueous phase was prepared by dissolving sodium lauryl sulfate (0.2 g) and glycerol (1.0 g) in distilled H₂O (15 mL) and heating the solution to 80 °C. The aqueous phase was gradually incorporated into the oil phase with constant stirring until a stable emulsion was achieved. The resulting mixture was allowed to cool to room temperature under stirring to yield the final ointment.

2.10 In vivo imiquimod (IMQ)-induced psoriasis-like murine assay

Sixty male BALB/c mice (6 weeks old) were obtained from SPF (Beijing) Biotechnology Co., Ltd. (Beijing, China). All animal experimental procedures complied with the "Guide for the Care and Use of Laboratory Animals" (National Institutes of Health Publication, revised 1996, No. 86-23, Bethesda, MD, USA) and were approved by the Institutional Animal Care and Use Committee (IACUC) of Hainan University (Approval no. HPIACUC2024087). Following a 1-week acclimation period, the mice were weighed and randomized into 6 groups (n = 10 per group): control (vehicle cream); IMQ model; calcipotriol (20 μg/g [positive control]); 10% *E. prostrata*-EA ointment; 2% WDL ointment; and 5% WDL ointment. Psoriasis-like dermatitis was induced by topical application of 62.5 mg of 5% IMQ cream (Hubei Keyi Pharmaceutical Co., Ltd., Hubei, China) to the shaved dorsal skin once daily for 7 consecutive d. Treatments were applied topically once daily for 7 d. Psoriasis severity was evaluated daily using

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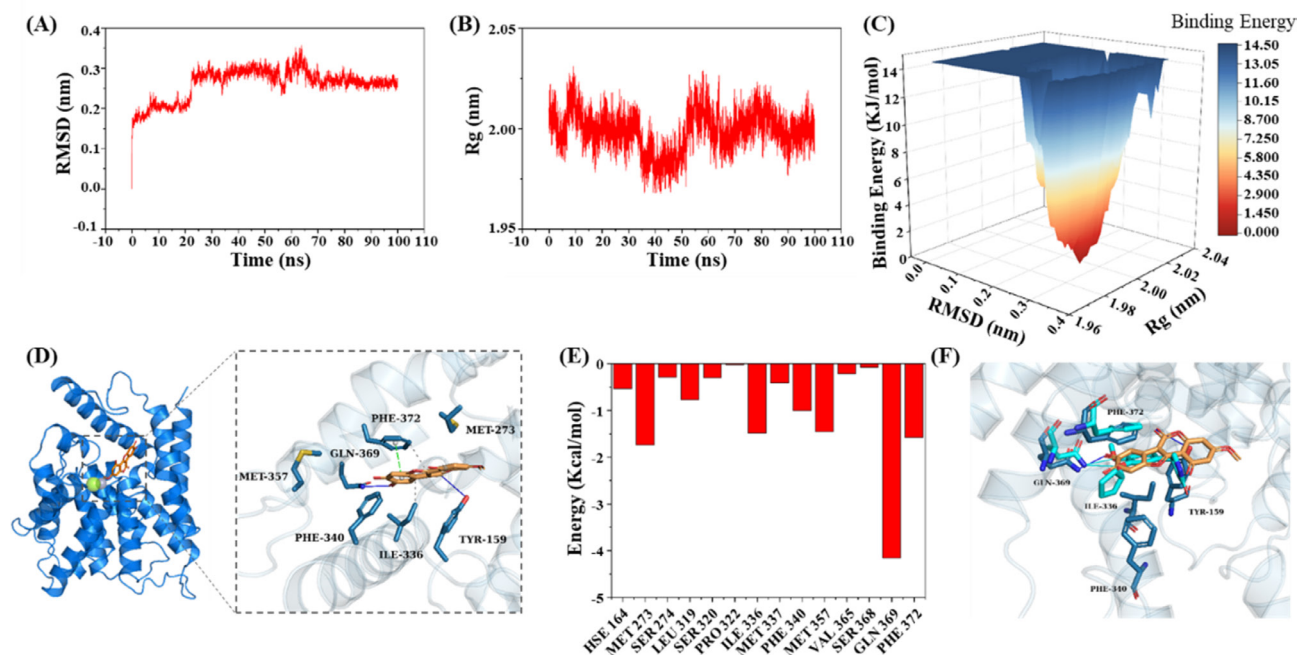


Figure 2 | MD simulation of the WDL-PDE4D complex, showing stable binding. (A) RMSD of the complex; (B) Rg of the complex; (C) FEL contour map; (D) The putative binding pattern between WDL and PDE4D; (E) The key residues identified by binding energy decomposition; (F) Structural alignment of the putative PDE4-WDL complex with the crystalized PDE4-rolipram complex (PDB ID: 1XON).

the Psoriasis Area and Severity Index (PASI), scoring erythema, scaling, and skin thickness on a scale of 0–4. On d 8, the mice were sacrificed and blood and skin samples were collected for biochemical analysis and histologic examination.

2.11 Subacute toxicity assay

Ten male and 10 female BALB/c mice (6 weeks old) were obtained from SPF (Beijing) Biotechnology Co., Ltd. All animal experimental procedures were performed in accordance with the “Guide for the Care and Use of Laboratory Animals” and approved by the IACUC of Hainan University (Approval no. HPIACUC2025002). Following a 1-week acclimation period, mice were weighed, randomized into 2 groups (5 males and 5 females per group), and shaved. The control group was treated daily with the cream vehicle, while the treatment group was treated daily with 15% WDL cream. The skin condition, food intake, and body weight were monitored throughout the study. After d 14 of treatment, serum samples were collected and the heart, liver, spleen, lungs, kidneys, and dorsal skin were harvested for weighing and H&E staining.

2.12 Biochemical analysis

Serum levels of cyclic adenosine monophosphate (cAMP), inflammatory cytokines (TNF- α , IL-6, IL-17A, and IL-23), aspartate transaminase (AST), and urea were

measured using ELISA kits (Byabscience, Wuhan, Hubei, China), according to the manufacturer’s instructions.

2.13 Histologic and immunohistochemical analysis

Skin samples were fixed in 10% formalin, embedded in paraffin, and sectioned at a thickness of 5 μ m. The sections were stained with H&E for histopathologic examination. Epidermal thickness was measured using ImageJ software (National Institutes of Health, Bethesda, MA, USA). Immunofluorescence staining was performed to detect TNF- α expression and CD3⁺ T-cell infiltration using specific antibodies (Abcam, Cambridge, UK). Fluorescence images were captured using a confocal microscope (Nikon Eclipse E100, Tokyo, Japan) and imaging system (Nikon Ds-U3).

3. RESULTS

3.1 Identification of WDL as a PDE4 inhibitor from *E. prostrata*

The methanolic extract of *E. prostrata* (*E. prostrata*-MeOH) was identified based on a systematic screening of in-house methanolic crude library, which was assembled from 1200 Chinese medicinal plants. The methanolic extract of *E. prostrata* (*E. prostrata*-MeOH) exhibited notable PDE4 inhibitory activity,

Table 3 | Predicted free energy (kcal/mol) for binding of WDL to PDE4D.

Energy terms	PDE4D-WDL (kcal/mol)
ΔG_{vdW}^a	-35.33 ± 0.07
ΔG_{ele}^b	-8.38 ± 1.98
ΔG_{MM}^c	-43.71 ± 2.06
$\Delta G_{pol, sol}^d$	42.42 ± 1.71
$\Delta G_{nonpol, sol}^e$	-3.51 ± 0.02
ΔG_{sol}^f	38.91 ± 1.73
ΔG_{bind}^g	-4.80 ± 2.62

^a ΔG_{ele} is electrostatic interactions calculated by the MM force field.
^b ΔG_{vdW} is van der Waals contributions from MM.
^c $\Delta G_{MM} = \Delta G_{vdW} + \Delta G_{ele}$.
^d $\Delta G_{pol, sol}$ is the polar contribution to solvation.
^e $\Delta G_{nonpol, sol}$ is the non-polar contribution to solvation.
^f $\Delta G_{sol} = \Delta G_{pol, sol} + \Delta G_{nonpol, sol}$.
^g $\Delta G_{bind} = \Delta G_{MM} + \Delta G_{sol}$ (absent of entropic contribution).

achieving 95.2% and 49.5% inhibition at 10 and 1 µg/mL, respectively (Figure 1A and Table 2). *E. prostrata*-MeOH was successively partitioned with PE, EA, and H₂O to obtain *E. prostrata*-PE, *E. prostrata*-EA, and *E. prostrata*-H₂O, respectively, to further investigate the active polar fractions responsible for PDE4 inhibition. *E. prostrata*-EA retained the highest PDE4 inhibitory activity, achieving 56.6% inhibition at 1 µg/mL (Table 2).

Surprisingly, the HPLC profiling of the *E. prostrata*-EA fraction revealed a relatively simple composition with only 2 major peaks observed at 254 nm, as shown in Figure 1B. These two predominant compounds were subsequently isolated and purified. Structural elucidation using ¹H NMR spectroscopy and HRMS identified the compounds as demethylwedelolactone [DMWDL] (compound 1) and WDL (compound 2; Figure 1C) when compared to literature data [30, 31].

A methylated derivative (compound 3) of WDL was synthesized and 4 commercially available analogs were obtained to explore the structure-activity relationships (SAR) of these compounds: isodemethylwedelolactone (compound 4) [32]; coumestrol (compound 5) [33]; neoglycyrol (compound 6) [34]; and P168140 (compound 7) [35] (Figure 1D). These compounds were tested for inhibitory activity against PDE4D. Specifically, over-methylation (compound 3), alteration in the positions or number of hydroxyl groups (compounds 4 and 5), and addition of aliphatic chains (compounds 6 and 7) all resulted in decreased activity compared to DMWDL and WDL (Table 2). These findings suggest that the presence and positions of hydroxyl groups, as well as the overall molecular framework of WDL, are critical for PDE4 inhibition.

Considering the high content of WDL in the *E. prostrata*-EA fraction, the potent PDE4 inhibitory activity,

and selection through the separation and extraction process for drug development, WDL was chosen for further evaluation.

3.2 MD simulation validates stable binding of WDL to PDE4D

Molecular docking was performed to obtain the PDE4D-WDL complex structure, which was subsequently subjected to a 100 ns MD simulation, to determine the binding pattern of WDL with PDE4D. The root means square deviation (RMSD) and radius of gyration (Rg) analysis of the PDE4D-WDL complex showed that the system quickly reached an equilibrium state and remained stable throughout the simulation, as shown in Figure 2A and B. In addition, the free energy landscape (FEL) contour map (Figure 2C) revealed a single low-energy state, suggesting a highly stable binding conformation of WDL with PDE4.

Based on these observations, the binding pose of WDL with PDE4D was generated by an average of 100 snapshots from the last 1 ns MD trajectories. The large conjugated scaffold of WDL was sandwiched by the hydrophobic residues of Phe372 on one side and Phe340/Ile336 on the other side and the two oxygen atoms formed two H-bond with residue Gln369, as shown in Figure 2D. These stacking and H-bond interactions were characteristic among PDE inhibitors, which could be the main driving force for the binding. In addition, the carbonyl oxygen of WDL formed an additional H-bond with Tyr159, further improving the binding affinity of WDL with PDE4D.

Alternatively, the interactions between of WDL-PDE4D could also be analyzed by the binding free energy (ΔG_{bind}) predicted using the molecular mechanism/Poisson-Boltzman surface area (MM-PBSA) method. ΔG_{bind} was decomposed into single-residue contributions and identified key residues, such as Met273, Ile336, Phe340, Gln369, and Phe372, as shown in Figure 2E. The van der Waals/hydrophobic interactions were the predominant force facilitating binding of WDL (Table 3). These predicted energy analyses were generally consistent with the observed interactions between WDL and PDE4D. Furthermore, structural alignment of the putative PDE4-WDL complex with the crystallized PDE4-rolipram complex revealed similar binding patterns, especially in terms of conserved π -stacking and H-bond interactions. However, the methyl and cyclopentyl moieties of rolipram were shown to engage in additional interactions near Gln369, which potentially contributed to the enhanced potency (Figure 2F). These observations may guide further structural optimization of WDL to improve binding affinity.

3.3 Cytotoxicity and *in vitro* anti-psoriatic effects in M5-induced HaCaT cells

HaCaT cell cytotoxicity was determined to evaluate the therapeutic potential of *E. prostrata*-EA and WDL on psoriatic keratinocytes. *E. prostrata*-EA exhibited

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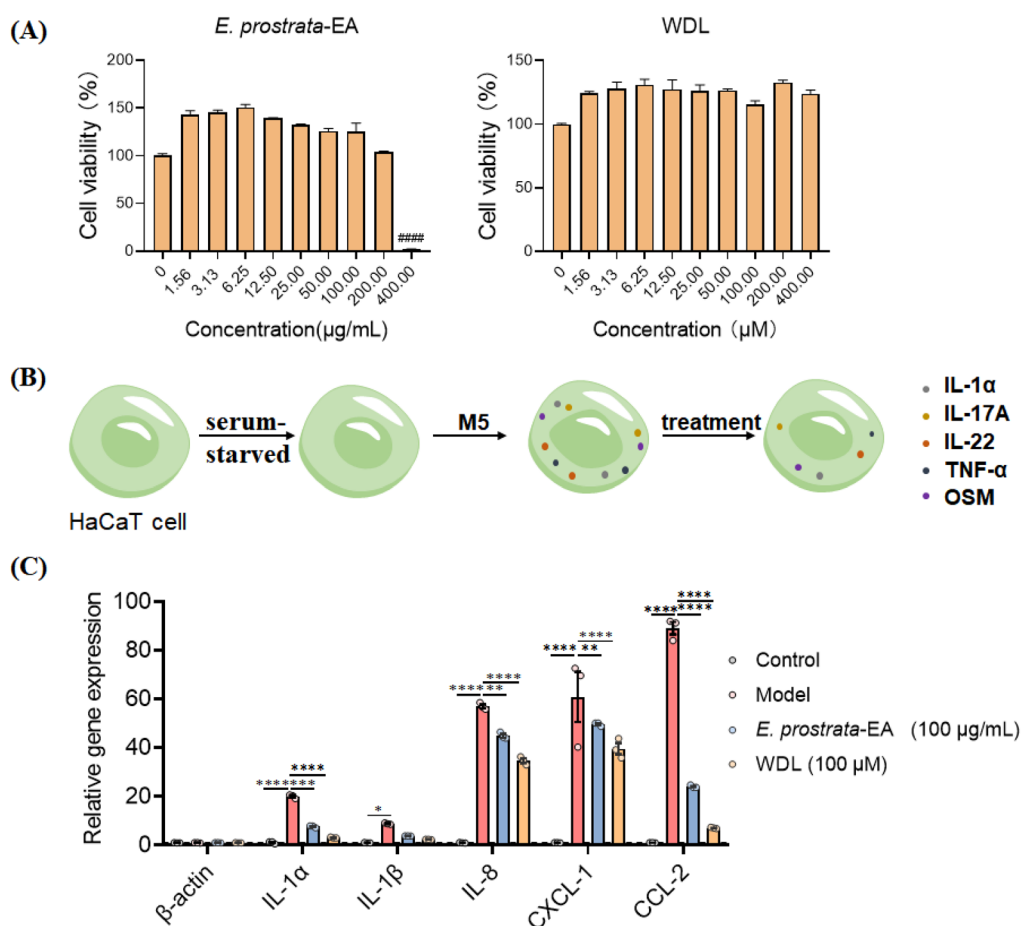


Figure 3 | *E. prostrata*-EA and WDL effectively inhibited inflammation in HaCaT cells. (A) Cytotoxicity assessment of *E. prostrata*-EA and WDL on HaCaT cells after 72 h of treatment; (B) Schematic illustration of the cell experimental design; (C) RT-qPCR results for the inflammatory factors (IL-1α, IL-1β, IL-8, CXCL-1, and CCL-2) at 24 h. n = 3 independent experiments and data are presented as the mean ± SEM. #####P < 0.0001 compared to the 0 μg/mL group; *P < 0.05, **P < 0.01, *P < 0.001, and ****P < 0.0001 compared to the control group**

minimal cytotoxicity at concentrations up to 200 μg/mL after 72 h of treatment but significant cytotoxic effects were observed at higher concentrations. Meanwhile, WDL did not exhibit detectable cytotoxicity at concentrations up to 400 μM (125.7 μg/mL; **Figure 3A**).

Subsequently, the anti-inflammatory effects of *E. prostrata*-EA and WDL were determined in an M5-induced HaCaT cell model, which mimicked the inflammatory environment of psoriatic skin. Treatment with WDL at 100 μM (31.42 μg/mL) significantly down-regulated pro-inflammatory cytokine (IL-1α, IL-1β, IL-8, CXCL-1, and CCL-2) mRNA expression to a greater extent than *E. prostrata*-EA at 100 μg/mL (**Figure 3B,C**). WDL not only exhibited superior PDE4 inhibitory activity but also demonstrated enhanced anti-inflammatory effects *in vitro*, highlighting the potential as a more efficacious therapeutic agent for psoriasis than *E. prostrata*-EA.

3.4 *In vivo* anti-psoriatic effects in IMQ-induced mice with psoriasis

The therapeutic potential of *E. prostrata*-EA and WDL was evaluated using an IMQ-induced psoriasis murine model (**Figure 4A**). Calcipotriol was selected as a positive control over the PDE4 inhibitor, apremilast, due to the systemic side effects of apremilast, including gastrointestinal disturbances and depression, as well as the comparatively limited market presence in topical formulations. Mice treated with topical ointments containing 10% *E. prostrata*-EA, 2% WDL, or 5% WDL exhibited significantly reduced psoriatic symptoms compared to the model group. Visual examination of the dorsal skin on d 7 showed that all treatment groups exhibited marked improvements in erythema, scaling, and epidermal thickness compared to the untreated IMQ model group (**Figure 4B**). The PASI scores showed a dose-dependent therapeutic effect with 5% WDL achieving the most significant reductions across all metrics (**Figure 4C**). Also,

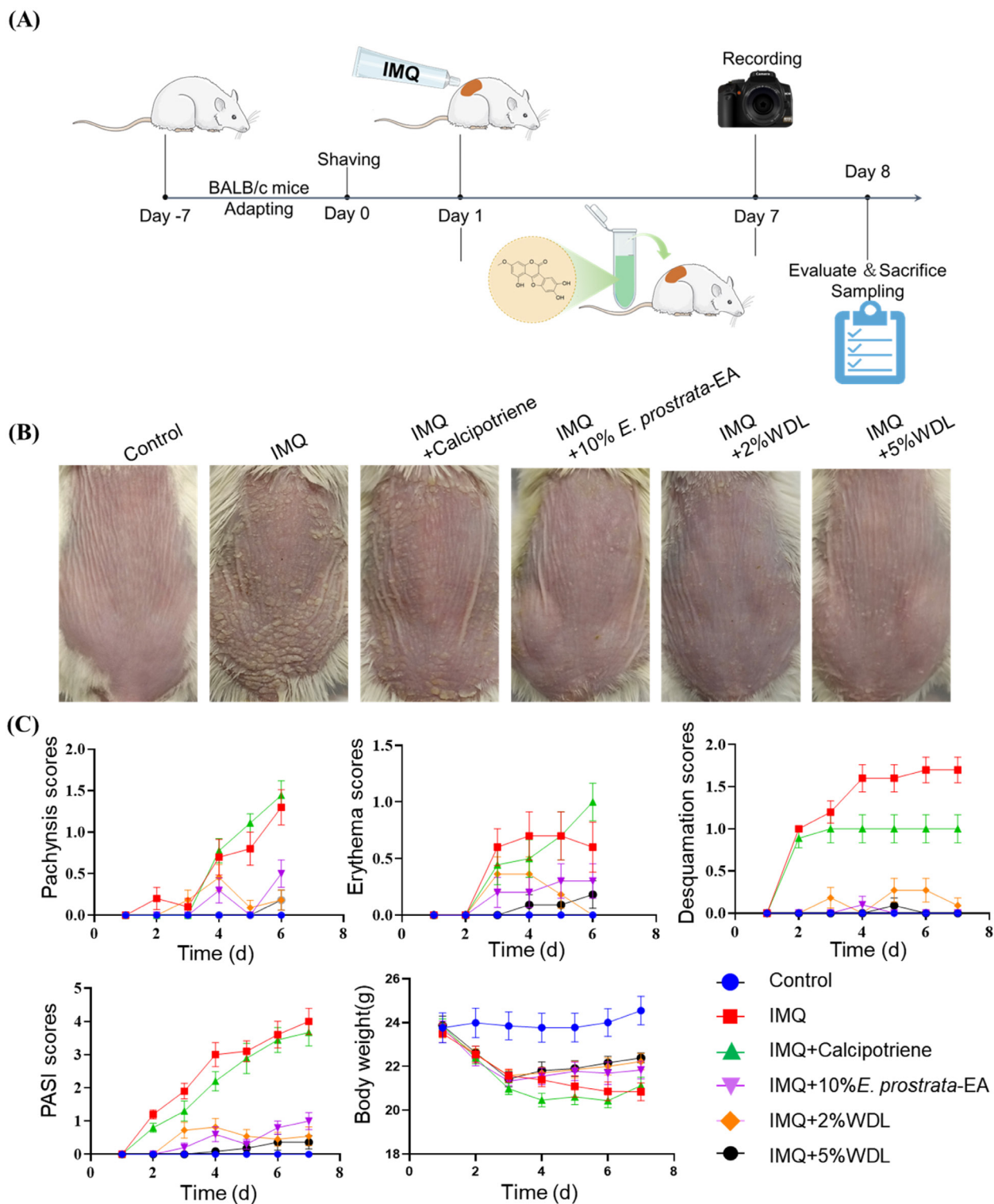


Figure 4 | *E. prostrata*-EA and WDL effectively alleviated psoriatic dermatitis *in vivo*. (A) Schemes of the IMQ-induced psoriatic murine model. (B) Representative images of lesions on d 7. (C) Pachynsis, desquamation, and erythema of the dorsal skin were scored daily from 0–4. The cumulative score, PASI score, and the sum of erythema, scale, and thickness scores. (D) The body weight of mice. $n = 10$ independent experiments and data are presented as the mean $\pm$ SEM.

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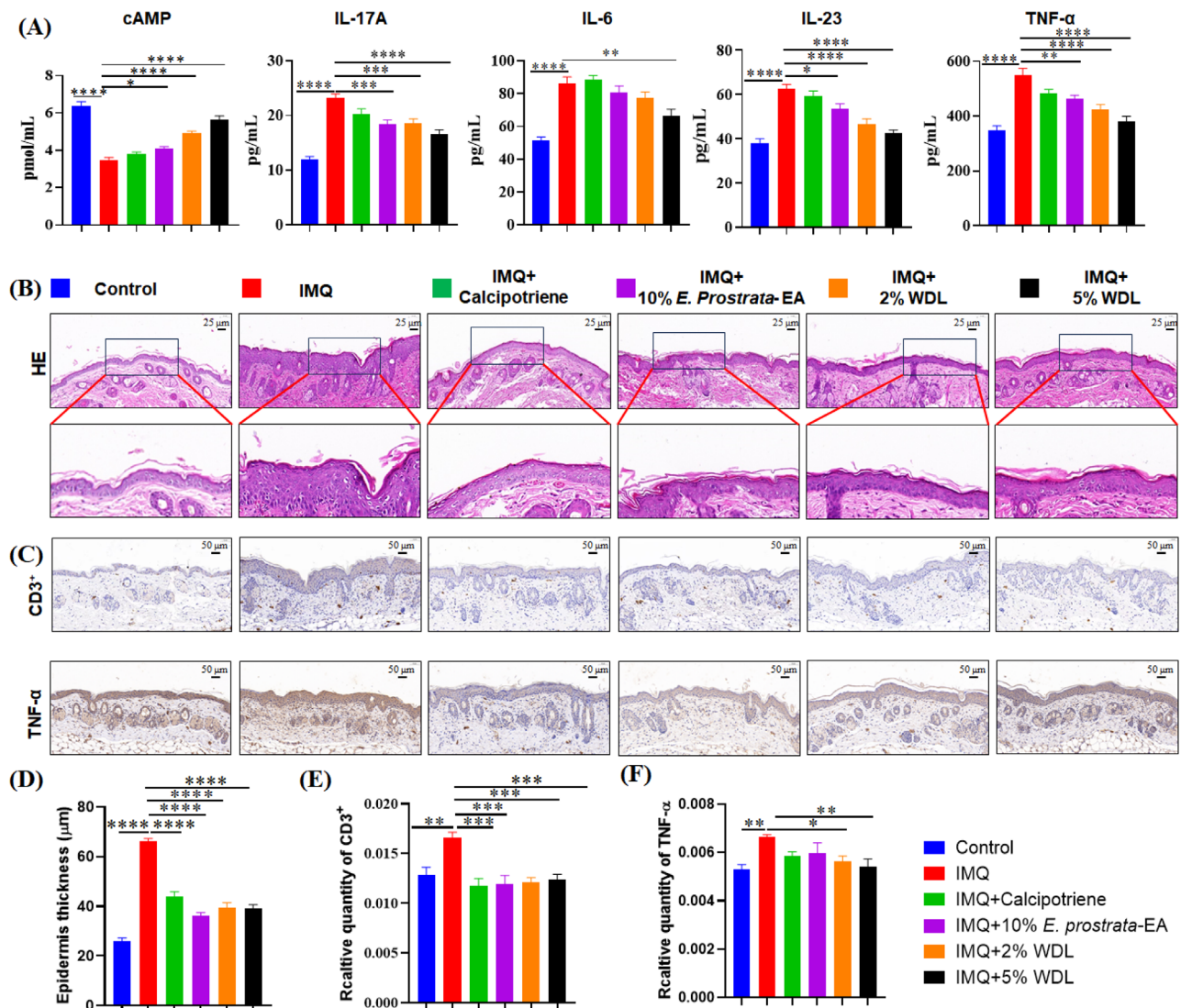


Figure 5 | *E. prostrata*-EA and WDL effectively increased cAMP and decreased the levels of several hallmark markers in mice with psoriasis. (A) ELISA quantification of cAMP, IL-17A, IL-6, IL-23, and TNF-α in skin samples; (B) Representative images of H&E staining (scale bar: 20 μm); (C) Representative images of immunohistochemistry for CD3⁺ T-cell infiltration and TNF-α expression (scale bar: 50 μm); (D) Quantification of stratum corneum thickness; (E-F) Quantification of CD3⁺ T-cell infiltration and TNF-α expression. Data are presented as the mean ± SEM, n = 10 independent experiments. *0.01 < P < 0.05, **0.001 < P < 0.01, ***P < 0.001, and ****P < 0.0001 compared to the control or IMQ group.

WDL demonstrated a better recovery in body weight compared to the model group (Figure 4C). Overall, the efficacy of all three treatment groups surpassed calcipotriol with 5% WDL demonstrating the best therapeutic effects.

In-depth cytokine analysis revealed that IMQ-induced mice exhibited decreased cAMP levels and elevated inflammatory cytokines, such as IL-17A, IL-6, IL-23, and TNF-α. Both *E. prostrata*-EA and WDL restored cAMP levels and significantly suppressed inflammatory cytokines (Figure 5A). The 5% WDL ointment demonstrated superior efficacy and nearly completely normalized the

cytokine profiles in agreement with the potent PASI score reductions.

H&E staining revealed that IMQ application induced characteristic psoriatic lesions, including epidermal hyperplasia and parakeratosis (Figure 5B). Treatment with *E. prostrata*-EA or WDL ointment markedly reduced the severity of these pathologic changes. Quantitative analysis showed that epidermal thickness, which was significantly increased in the IMQ model group due to abnormal keratinocyte proliferation, was restored to near-normal levels following treatment (Figure 5D). Immunofluorescence staining further demonstrated

Table 4 | Metabolic stability of WDL in human and mouse liver microsomes.

Compounds	HLM ^a		MLM ^a	
	$t_{1/2}^b$ (min)	Remaining (% , $t = 60$ min)	$t_{1/2}^b$ (min)	Remaining (% , $t = 60$ min)
Verapamil	17.54	8.91	7.20	0.33
WDL	111.58	71.19	12.41	3.73

^aHLM: human liver microsome stability, MLM: mice liver microsome stability.

^b $t_{1/2}$: half-life.

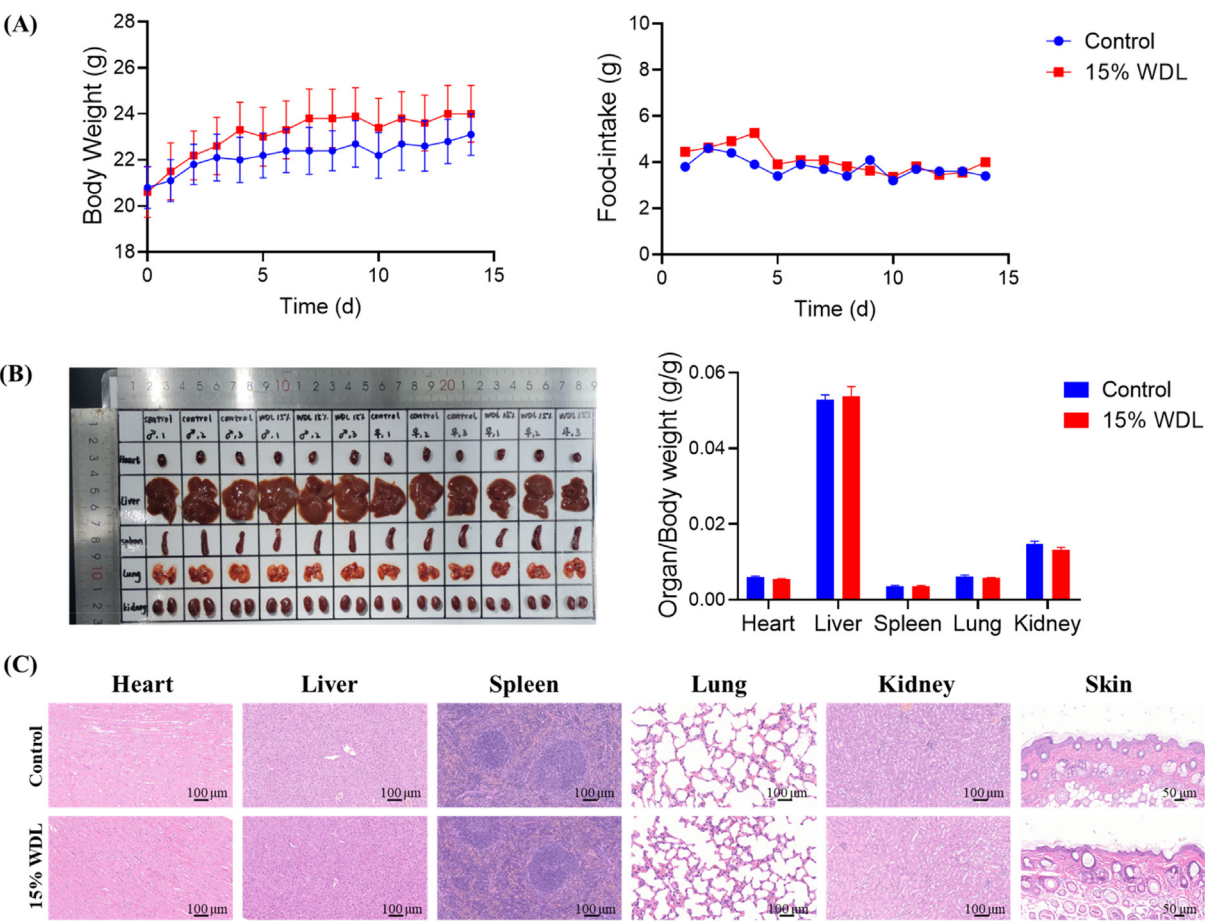


Figure 6 | The 15% WDL ointment was non-irritating, safe, and non-toxic to mice. (A) Body weight and food-intake of mice; (B) Representative images of the major organs and organ-to-body ratios in mice; (C) Histologic representative images of the heart, liver, spleen, lungs, kidneys, and skin of the mice were captured after administration of control and 15% WDL for 14 d; Data are presented as the mean $\pm$ SEM ($n = 10$ independent experiments).

that TNF- α expression and CD3⁺ T-cell infiltration were significantly elevated in the IMQ group compared to the control group, reflecting a robust inflammatory response. Both *E. prostrata*-EA and WDL treatment significantly suppressed the TNF- α level and CD3⁺ T-cell infiltration in the epidermis (Figure 5C,E, & F). Five percent WDL ointment achieved the greatest reduction in inflammatory markers, highlighting the superior anti-inflammatory efficacy.

3.5 Metabolic stability of WDL in liver microsomes

The metabolic stability of WDL was assessed in human liver microsomes (HLMs) and mouse liver microsomes (MLMs) with verapamil used as a reference compound. WDL exhibited species-specific differences in metabolic degradation. WDL demonstrated high stability with a half-life ($t_{1/2}$) of 111.58 min and 71.19% of the compound remaining after 60 min in HLM (Table 4).

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In contrast, a faster metabolic rate was noted in MLM with a $t_{1/2}$ of 12.41 min and only 3.73% remaining at 60 min (Table 4). These results suggested that WDL has favorable metabolic stability in human microsomes, supporting the potential of WDL for further drug development.

3.6 Subacute toxicity evaluation of WDL

Subacute toxicity study was conducted using BALB/c mice to further assess the safety profile of WDL as a potential therapeutic candidate for psoriasis. The animals were treated with a topical formulation of 15% WDL (3 times the effective therapeutic dose) for 14 consecutive d (Supporting Information, Figure S4A). Throughout the experimental period, no significant changes were noted in body weight or food intake between the treatment and control groups, suggesting no systemic toxicity (Figure 6A). Biochemical analysis revealed that the serum AST and urea levels in WDL-treated mice remained within normal physiologic ranges, indicating no detectable hepatotoxicity or nephrotoxicity (Supporting Information, Figure S4B). Additionally, macroscopic examination of major organs, including the heart, liver, spleen, lungs, and kidneys, showed no abnormalities in morphology, coloration, or organ-to-body weight ratios (Figure 6B). A histopathologic evaluation using H&E staining confirmed the absence of pathologic alterations in critical organs, including the liver, kidneys, lungs, spleen, heart, and skin (Figure 6C). Collectively, these findings suggested that topical application of 15% WDL did not elicit significant systemic or organ-specific toxicity, supporting a favorable safety profile for further preclinical development.

4. DISCUSSION

In the current study 1200 Chinese medicinal plant extracts were systematically screened and identified *Eclipta prostrata* as a potent PDE4 inhibitor, highlighting the potential of *Eclipta prostrata* as a source of novel anti-inflammatory agents. Through bioassay-guided fractionation, the EA fraction (*E. prostrata*-EA) was shown to exhibit the highest PDE4 inhibitory activity, leading to the isolation of WDL as the principal bioactive compound. Although WDL has been previously reported for anti-inflammatory and hepatoprotective properties [36], the current study is the first study to establish the potent PDE4 inhibition of WDL and the therapeutic potential of WDL for psoriasis treatment.

MD simulations provided mechanistic insights into the interaction between WDL and PDE4D, revealing stable binding mediated by van der Waals forces and hydrogen bonding with key residues, such as Phe372 and Gln369. The binding free energy calculations further supported the strong affinity of WDL for PDE4D, in agreement with the robust inhibitory activity observed in enzymatic assays. These findings not only validated WDL as a PDE4 inhibitor but also provided a molecular

framework for future structure-based optimizations to enhance the potency and drug-like properties of WDL.

WDL significantly suppressed the expression of pro-inflammatory cytokines (IL-1 α , IL-1 β , and IL-8) and chemokines (CXCL-1 and CCL-2) in M5-induced HaCaT cells *in vitro*, a widely used psoriasis model. These findings indicated that WDL effectively modulates key inflammatory pathways implicated in the pathogenesis of psoriasis. The superior efficacy of WDL compared to *E. prostrata*-EA further confirmed the role of WDL as the primary active component contributing to the anti-inflammatory effects of the extract.

The therapeutic potential of WDL was further validated in an IMQ-induced psoriasis-like murine model. Topical application of WDL significantly alleviated psoriatic symptoms, including erythema, scaling, and epidermal hyperplasia, with greater efficacy than calcipotriol. Histologic and immunohistochemical analyses confirmed that WDL reduced epidermal thickening, inflammatory cell infiltration, and pro-inflammatory cytokine expression. Notably, WDL treatment restored cAMP levels and downregulated key cytokines (TNF- α , IL-6, IL-17A, and IL-23), demonstrating the systemic anti-inflammatory effects via PDE4 inhibition. In the current study calcipotriol was selected as the positive control owing to widespread use and established topical efficacy in the clinical treatment of psoriasis. Although apremilast is a representative PDE4 inhibitor approved for systemic use, WDL is associated with adverse effects, such as gastrointestinal discomfort and mood disturbances, and WDL is not currently formulated for topical application. Therefore, WDL may offer a safer and more convenient alternative for topical therapy.

In addition to promising *in vivo* efficacy, WDL demonstrated favorable metabolic stability in HLM, suggesting good intrinsic stability. Importantly, the subacute toxicity evaluation indicated that WDL exhibits a favorable safety profile. Even at a 3-fold higher effective dose, WDL did not induce significant systemic toxicity, as evidenced by stable body weight, normal organ morphology, and unaltered biochemical markers of hepatic and renal function. These results suggested that WDL has strong potential as a safe and effective topical therapy for psoriasis.

The novelty of the current study lies in the identification of WDL as a potent PDE4 inhibitor with promising anti-psoriatic effects, establishing WDL as a strong candidate for topical drug development. Unlike systemic PDE4 inhibitors, such as apremilast, which are associated with gastrointestinal and neuropsychiatric side effects, topical application of WDL offers a localized therapeutic approach with minimal systemic exposure. Furthermore, derivation of WDL from a well-known traditional medicinal plant enhances accessibility, cost-effectiveness, and sustainability compared to biologic therapies.

Overall, the current study has provided compelling evidence that WDL is a promising lead compound for the development of novel topical PDE4 inhibitors for

psoriasis treatment. Future studies should focus on optimizing WDL formulations, evaluating long-term efficacy and safety, and conducting clinical trials to establish its therapeutic potential in human patients.

ETHIC STATEMENT

All animal experimental procedures complied with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication, revised 1996, No. 86-23, Bethesda, MD, USA) and were approved by the Institutional Animal Care and Use Committee (IACUC) of Hainan University (Approval no. HPIACUC2024087).

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SUPPLEMENTARY MATERIAL

Supplementary Material can be downloaded from https://amm-journal.org/wp-content/uploads/2025/05/amm20250017_Suppl.pdf.

CONFLICT OF INTEREST

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

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