

# Essential Oil of *Matricaria chamomilla* Alleviate Psoriatic-Like Skin Inflammation by Inhibiting PI3K/Akt/mTOR and p38MAPK Signaling Pathway

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**Background:** The traditional *Matricaria chamomilla* L. has been used to treat dermatitis for thousands of years. Due to emerging trends in alternative medicine, patients prefer natural remedies to relieve their symptoms. Therefore, finding safe and effective plant medicines for topical applications on the skin is an important treatment strategy for dermatologists. German chamomile (*Matricaria chamomilla* L.) from the Compositae family is a famous medicinal plant, often known as the “star of medicinal species.” However, the function of *Matricaria chamomilla* essential oil on skin inflammation has not been thoroughly examined in earlier research.

**Methods:** GC-MS analyzed the components of MCEO, and this study explored the anti-inflammation effects of MCEO on psoriasis with network pharmacological pathway prediction. Following this, we used clinical samples of psoriasis patients to confirm the secretory characteristic of relative inflammatory markers. The therapeutic effect of MCEO on skin inflammation was detected by examination of human keratinocytes HaCaT. At the same time, we prepared imiquimod-induced psoriatic-like skin inflammation in mice to investigate thoroughly the potential inhibition functions of MCEO on psoriatic skin injury and inflammation.

**Results:** MCEO significantly reduced interleukin-22/tumor necrosis factor  $\alpha$ /lipopolysaccharide-stimulated elevation of HaCaT cell inflammation, which was correlated with downregulating PI3K/Akt/mTOR and p38MAPK pathways activation mediated by MCEO in HaCaT cells treated with IL-22/TNF- $\alpha$ /LPS. Skin inflammation was evaluated based on the PASI score, HE staining, and relative inflammatory cytokine levels. The results showed that MCEO could significantly contribute to inflammatory skin disease treatment.

**Conclusion:** MCEO inhibited inflammation in HaCaT keratinocytes induced by IL-22/TNF- $\alpha$ /LPS, the potential mechanisms associated with inhibiting excessive activation and crosstalk between PI3K/Akt/mTOR and p38MAPK pathways. MCEO ameliorated skin injury in IMQ-induced psoriatic-like skin inflammation of mice by downregulating the levels of inflammatory cytokines but not IL-17A. Thus, anti-inflammatory plant drugs with different targets with combined applications were a potential therapeutic strategy in psoriasis.

**Keywords:** *Matricaria chamomilla*, essential oil, psoriasis, HaCaT, IMQ, mouse

## Introduction

Psoriasis is a chronic immune-mediated disease influencing 100 million individuals worldwide. Hence, action is needed to provide care for patients with psoriasis and eliminate discrimination.<sup>1</sup> It is associated with multiple comorbidities and seriously affects patients' quality of life. It is mainly manifested as epidermal hyperplasia, abnormal keratinocyte differentiation, marked Artery dilatation, and marked seep inflammatory cells around dermal capillaries in psoriatic histomorphology. The pathogenesis of psoriasis is complex and remains unelucidated. Stimulation of keratinocytes has

been considered to lead to the generation of secondary mediators, for example, cell factors, chemokines, and antibacterial peptides. Thus, psoriasis can be induced by the dysregulation of immune and keratinocyte differentiation accompanied by persistent inflammation.<sup>2,3</sup> At present, in addition to traditional immunosuppressants, for example, methotrexate (MTX) and cyclosporine, new therapeutic advances, including selective inhibitors, such as Phosphodiesterase 4 (PDE4) inhibitor, Janus kinase (JAK) inhibitor, and mAbs, anti-cytokine/T cell therapy, and relative gene therapy, was applied to clinical psoriasis.<sup>3,4</sup> However, with the increase in the frequency of clinical use of biological agents for patients with prolonged drug use, the clinical effects of biological agents may be reduced. Furthermore, various adverse reactions limit their clinical application.<sup>5</sup> Also, as the cost of psoriasis treatment is a major challenge, we require efficient, safe, and cost-effective therapies.

Despite advances in drug application, natural herbal alternative therapy has become a key factor for skin disease therapy due to the increasing demand for herbs, associated low costs, and the continuing adverse effects of modern medicines.<sup>6</sup> Moreover, herbal remedies, especially those derived from plants, are effective against various skin conditions.<sup>7</sup> Some investigations have found that natural plant extracts from Aloe vera, *Mahonia aquifolium*, *Matricaria recutita*, *Silybum marianum*, *Gaultheria procumbens*, special populations are effective against a variety of skin conditions.<sup>7</sup>

German chamomile (*Matricaria chamomilla* L.) from the Compositae family is a famous medicinal plant called “a star of medicinal species.” These herbs have been used in Greece, Rome, and ancient Egypt for thousands of years. Dai et al found that there are many kind of chemical constituents in *German chamomile*, including organic acids, flavonoids, coumarins, volatile oil, monoterpenes, sesquiterpenes, diterpenes and triterpenes ect.<sup>8</sup> *German chamomile* has definite therapeutic effects on treating stomach problems, cramps, dermatitis, minor infections,<sup>8–12</sup> bactericidal and muscle-relaxant activities. In addition, some animal studies have certified anti-anxiety,<sup>13</sup> anti-cancer, cholesterol-lowering, wound-healing,<sup>14</sup> anti-diabetic properties<sup>15</sup> of this plant. However, its application in the field of inflammatory skin diseases is just emerging. Lin et al showed that besides anti-inflammatory and antioxidant, *M. chamomilla* essential oil (MCEO) can promote wound healing and repair the skin barrier.<sup>9</sup> Moreover, Wang et al found that MCEO decreased the proinflammatory factors (TNF- $\alpha$  and IL-6) to inhibit the Th17 cell differentiation during eczema.<sup>16</sup> In China and Japan, it was usually used externally in the skin field, including anti-itch and anti-inflammatory;<sup>17</sup> Sun protection,<sup>18</sup> relieves skin irritation<sup>19</sup> and antiviral or prevents viral diseases.<sup>20</sup> To date, no experimental reports on the application of *German chamomile* in psoriasis have been published.

Network pharmacology is a new strategy to systematically study the relationship between diseases, drugs, and targets.<sup>21</sup> A growing body of evidence supports network pharmacology as an effective tool for studying the molecular mechanisms of herbs containing complex multi-compounds.<sup>22</sup> The pharmacological effects and potential targets of MCEO on psoriasis were systematically discussed utilizing network pharmacology. The anti-inflammatory effect of MCEO on psoriatic-like skin inflammation was validated in vivo and in vitro based on network pharmacological prediction. In addition, Luminex evaluated the levels of relative inflammatory cytokines in psoriatic patients to identify potential targets for the anti-inflammatory effects of MCEO in humans. According to the analysis results of network pharmacology, we evaluated the anti-inflammation effects and regulating mechanism of MCEO in interleukin-22 (IL-22)/tumor necrosis factor  $\alpha$  (TNF- $\alpha$ )/lipopolysaccharide (LPS)-stimulated human keratinocytes (HaCaT) in vitro and in the rodent imiquimod (IMQ)-induced psoriatic skin inflammation model mice, to clarify the potential value of MCEO in clinical application of inflammatory skin diseases from the perspective of anti-inflammatory.

## Materials and Methods

### MCEO Extraction and GC-MS Analysis

MCEO was obtained by steam distillation of flowers from *Matricaria chamomilla*. Qualitative analyses of MCEO were carried out by Gas Chromatography(GC)-mass spectrometry (MS) using a 7890A-5975C type GC-MS (Agilent Technology Co., LTD, California, USA). Chromatographic conditions: HP-5MS quartz capillary column (30m $\times$ 0.25mm $\times$ 0.25 $\mu$ m), inlet temperature 250°C; The carrier gas was helium (99.999%), the shunt ratio was 10:1, and the sample size was 1 $\mu$ L. Mass spectrometric conditions: electron bombardment ion source (EI), ion source

temperature 230°C, interface temperature 280°C; The electron energy is 70eV, the four-pole temperature is 150°C, and the mass scanning range is 20–650Amu. Heating procedure: the initial temperature is 60°C; keep it for 5min, and rise to 280°C at the rate of 5°C/min. Hold for 10 minutes.

## Analysis of Network Pharmacology

The MCEO network was constructed with MCEO compounds. According to the results of GC-MS analysis, Genes related to MCEO compounds were searched with PubChem (<https://pubchem.ncbi.nlm.nih.gov/>) and target genes using a compound database. Psoriasis-related genes were gathered using GeneCards (<http://www.genecards.org/>) with the keyword “psoriasis.” Common genes of the MCEO network and psoriasis-related gene set were organized. A protein-protein interaction network (PPIN) was constructed with target genes between MCEO and psoriasis with the STRING database (<https://string-db.org/>) and then visualized by Cytoscape (3.9.1). DAVID (<https://david.ncifcrf.gov/>) database was used to perform Gene Ontology (GO) annotation analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of the targets. Psoriasis-related KEGG pathways and GO process were organized, and cut-off criteria were  $p$ -value < 0.05.

## Patient

### Patient Recruitment and Samples

A total of 31 psoriasis vulgaris patients, whose diagnoses were based on the Guideline for the diagnosis and treatment of psoriasis in China (2021 edition) and has not been treated with biological agents, were recruited from Taizhou Second People’s hospital (Zhejiang, China) from February 2021 to December 2021, along with 35 presenting non-inflammatory skin conditions as controls ([Supplement Table 1](#)).

Research work was approved by the Ethics Review Committee on Human Research of Taizhou Second People’s Hospital (tzey-ky202101) and executed based on the Declaration of Helsinki Principles. Taizhou Second People’s Hospital (Taizhou) recruited all participants in this study. All the patients signed informed consent forms.

Sterile venous blood was obtained by standard venepuncture and stored overnight in a 4°C refrigerator. The next day, we separated serum by centrifuging the blood samples at 1500g for 15 min at 4°C. All serum samples were kept frozen at –80 °C until assay.

## Inflammatory Cytokines Assay by Luminex in Clinical Sample

Serum multiple cytokine analysis was executed using Bio-Plex Pro Human Cytokine 27-plex (M500KCAF0Y, Bio-rad systems), measure the concentrations of Interleukin (IL)-1 $\beta$ , IL-10, IL-17A, IFN- $\gamma$  and Tumor Necrosis Factor- $\alpha$  (TNF- $\alpha$ ) in one sample at the same time, follow the manufacturer’s instructions.

## Chemicals, Antibodies, and Reagents

The following materials were used in the present study: MCEO (Jiaxing Hanjiangfangcao Bioengineering Co. Ltd., Zhangjiang, China); DMSO (20220813); FBS (10099-141C, Gibco, Australia); DMEM high glucose medium (Hyclone, USA); Penicillin-Streptomycin (Gibco, Australia); 0.25% trypsin-EDTA (Gibco, Australia); recombinant human TNF- $\alpha$  (R&D system, America); recombinant human IL-22 (R&D system, America); LPS (L2630, Sigma-Aldrich, Merck); Cell Counting Kit-8 (MedChemExpress, America); propidium iodide (BIO BASIC INC, Canada); PMSF, RIPAlysis buffer, BCA protein assay kit, BeyoECL Star, primary antibody dilution buffer, hematoxylin and eosin staining kit (Beyotime, China); multicolor prestained protein ladder (Shanghai Epizyme Biomedical Technology, China); Antibodies for Phospho-mTOR (Ser2448)(D9C2) Rabbit mAb #2475, mTOR (7C10) Rabbit mAb #2983, Phospho-p38 MAPK (Thr180/Tyr182) (D3F9) Rabbit mAb #4511, p38 MAPK (D13E1) Rabbit mAb #8690, Phospho-Akt (Ser473) (D9E) Rabbit mAb #4060, Akt (pan) (C67E7) Rabbit mAb #4691, Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) (D13.14.4E) Rabbit mAb #4370, p44/42 MAPK (Erk1/2) (137F5) Rabbit mAb #4695, Bcl-2 (124) Mouse mAb #15071, Caspase-3 Antibody #9662,  $\beta$ -Actin (D6A8) Rabbit mAb (HRP Conjugate) #12620, Anti-mouse IgG, HRP-linked Antibody #7076, Anti-rabbit IgG, and HRP-linked Antibody #7074 (Cell Signaling Technology, Danvers, MA, USA); Caspase-8 Antibody, Caspase-9 Antibody, Bax Antibody, Goat anti-Mouse IgG HRP (TS0002), and Goat anti-

Rabbit IgG HRP (TS0001)(Abmart Technology, Shanghai, China); Anti-PI 3 Kinase p85 alpha (phospho Y607) (ab182651), Anti-PI 3 Kinase p85 alpha antibody [6G10] (ab189403)(Abcam TechnologyCambridge, England); SAV HRP (Streptavidin-Horseradish Peroxidase Pre-diluted) (51–75477E) (BD Pharmingen); PrimeScript RT Reagent Kit (Perfect Real time, TaKaRa, Japan); SYBR Green Realtime PCR MasterMix (TOYOBO, Japan); Mouse ACTB Endogenous Reference Genes Primers (B661302), and Human ACTB Endogenous Reference Genes Primers (B661102) (Sangon Biotech, China); DEPC treated Water and TRIzol Reagent (Ambion, America).

## Cell Experiment

### General Cell Culture and Treatment

We obtained immortalized human HaCaT keratinocytes from Wuhan University Cell Bank (Wuhan, China). Dulbecco's modified Eagle's medium (DMEM), added into 10% fetal bovine serum (FBS), 100 µg/mL of streptomycin, and 100 U/mL of penicillin, were used for cell culture. Cells were incubated at 37°C with 5% CO<sub>2</sub>. We used rhIL-22 (20ng/mL), rhTNF-α (10ng/mL), or LPS (10µg/mL) to treat HaCaT cells. We prepared the stock solution of MCEO in dimethyl sulfoxide (DMSO, 0.1%v/v) and, according to experimental design, diluted the differential concentration in the medium for dealing with HaCaT cells. Control cells were treated with the same amount of DMEM (DMSO 0.1%v/v); this concentration of DMSO did not affect the cell viability.

For rhIL-22/rhTNF-α/LPS with or without MCEO treatment scheme, HaCaT cells were divided into normal control group, rhIL-22 (24h, 20ng/mL)/rhTNF-α (24h, 10ng/mL)/LPS (1h, 10 µg/mL) treatment group, and pre-MCEO (24h) +rhIL-22 (24h)/TNF-α (24h)/LPS (1h) treatment group. First, MCEO treated HaCaT cells for 24h, and then PBS washed the cells twice. Subsequently, rhIL-22 (20ng/mL, 24h), rhTNF-α (10ng/mL, 24h), or LPS (10µg/mL, 1h) were added, respectively. Cells were harvested and lysed for Western blot, and qRT-PCR was performed as described below.

### Cell Viability Assay

CCK-8 method was adopted to calculate the cell survival rate. HaCaT cells were grown in 96-well plates for 24h or 48h. Cells were harvested after they reached 50–60% confluence and were treated with MCEO for 24h and 48h. CCK-8 solution (10 µL) was added into each well and then incubated at 37 °C with 5% CO<sub>2</sub> for 2h. We used Multiskan Spectrum (Thermo Fisher Scientific, USA) to measure absorbance at 450 nm.

### Flow Cytometric Analysis

Cells ( $5 \times 10^5$ /plate) were seeded and attached in 60 mm culture dishes for 24h. The cells were harvested using trypsin after MCEO treatment for 24h. We used PBS to wash the collected cells and ethanol to fix them at 4 °C for 2h. We incubated fixed cells with RNase (10µg/mL) at 37 °C for 30min. Subsequently, PBS washed the fixed cells twice and then stained them with propidium iodide (PI, 10µg/mL) for 30 min in the dark. The cell cycle was monitored using CytoFLEX (BECKMAN CULTER, CA, USA).

## Animals

Female BALB/c mice (eight-to-nine-week-old; raised under specific pathogen-free (SPF) conditions) were purchased from Shanghai Vital River Laboratory Animal Technology Co., Ltd. (Shanghai, China). Mice were bred under constant temperature and humidity conditions with a 12-h light/dark cycle. Food and water are freely afforded. All experiment schemes were agreed upon by the Ethical Committees of Taizhou University (Taizhou, China) (TZXY-2022-20221027). All animal procedures were conducted in compliance with the Regulations for the Administration of Affairs Concerning Experimental Animals (1988.11.1), and humanely treated. Animals were anesthetized by isoflurane (Riward Life Science Co., Ltd, Shenzhen, China)(3% for induction; 1–3% for installation) in a mixture of 0.25% air and 0.5% O<sub>2</sub>.

BALB/c mice were divided into three groups (n=6) randomly: Olive oil group (Con), IMQ group (IMQ), and IMQ+MCEO group (IMQ+MCEO). MCEO (1:10 dilution of olive oil) was applied on the bare back for seven days in a row (twice a day, once in the morning and once in the afternoon; 200µL/time/mice). Subsequently, mice were administered a daily 62.5 mg of commercially available IMQ cream (5%) (Sichuan Mingxin Pharmaceutical Co. LTD, H20030128) on the bare back for seven days in a row, concurrently with MCEO treatment as mentioned

above. The control group and IMQ group were given similarly with a control cream (olive oil; fagron). On day eight, all mice were euthanized. Serum samples were collected, and the back skin was obtained for subsequent detection.

## Measurement of Indicators

### Scoring Severity of Skin Inflammation

Skin lesions were evaluated by Psoriasis Area and Severity Index (PASI). We calculated the overall score based on erythema, scaling, and thickening but excluding the affected skin area. According to the severity of each item, scored independently (from 0 to 4): 0, none; 1, mild; 2, general; 3, typical; 4, very specific. Inflammation severity was determined by cumulative score (erythema, scaling, and thickening) (scale 0–12).

### Histological Examination

We fixed the skin tissues using a 4% paraformaldehyde solution and embedded skin tissues in paraffin. After sectioning the paraffin-embedded tissues, hematoxylin and eosin (H&E) stain was performed for histological evaluation. An Olympus microscope under a 100× light microscope got pictures, and epidermal thickness was calculated by the Image J software (NIH Image, USA).

### Quantitative Real-Time PCR Assay (qRT-PCR)

The mRNA expressions of IL-6, IL-1 $\beta$ , TGF- $\beta$ , IL-10, TNF- $\alpha$ , IL-17A, and IL-22 in HaCaT cells and skin sections were detected using the qRT-PCR method. We used TRIzol<sup>®</sup> reagent to extract RNA and a reverse transcription kit (Takara Bio, Inc.) to synthesize cDNA. qPCR was operated using SYBR premixed EX Taq<sup>™</sup> cDNA and specific gene primers. We used the LightCycler480 system (Roche) to amplify target genes. We used the standardized ACTIN gene to determine the relative expression quantity of each gene, which was calculated via the  $2^{-\Delta\Delta C_q}$  method. The following primers were used: Human IL-6-forward, 5'-GACAGCCACTCACCT CTCAGAAC' and reverse, 5'-CCAGGCAAGTCTCCTCATTGAATCC'; Human IL-1 $\beta$ -forward, 5'-TACGAATCTCCGACCACCACTACAG' and reverse, 5'-ACACCACTTGTTGCTCCATATC CTG-3'; Human TGF- $\beta$ -forward, 5'-ACTACTACGCCAAGGAGGTCA CC' and reverse, 5'-TG AGGTATCGCCAGGAATTGTTGC-3'; Human IL-10-forward, 5'-GGACAACATACT GCTAAC CGACTCC-3' and reverse, 5'-CTTCACCTGCTCCACTGCCTTG-3'; Human IL-17A-forward, 5'-GGTGTCAGTCTACTGCTGCTG-3' and reverse, 5'-GGTCCTCATTGCGGTGGAGATTC -3'; Human ATCB-forward, 5'-GTGCTATGT TGCTCTAGACTTCG-3' and reverse, 5'-ATGCC ACAGGATTCCATACC-3'; Mouse IL-6-forward, 5'-CTTCTTGGGACTGATGCTGGTGAC-3' and reverse, 5'-CTCTCTGAAGGACTCTGGCTTTGTC-3'; Mouse IL-1 $\beta$ -forward, 5'-TTCAGG CAGGCAGTATCACTCATTG-3' and reverse, 5'-TGTCGTTGCTTGGTTCTCC TTGTAC-3'; Mouse TGF- $\beta$ -forward, 5'-ACCGCAACAACGCCATCTATGAG-3' and reverse, 5'-AGCCCTG TAT TCCGTCTCCTTGG-3'; Mouse IL-10-forward, 5'-GGACAACATACTGCTAACC GACTC C-3' and reverse, 5'-CTTCACCTGCTCCACTGCCTTG-3'; Mouse TNF- $\alpha$ -forward, 5'-CACCA CGCTCTTCTGTCTACTGAAC-3' and reverse, 5'-TGACGGCAGAGAGGAGGTTGAC-3'; Mouse IL-17A -forward, 5'-TGATGCTGTTGCTGCTGCTGAG -3' and reverse, 5'-GGTCTTCA TTGCGGTGGAGAGTC-3'; Mouse ATCB-forward, 5'-CCTGGCACCCAGCACAAAT -3', and reverse, 5'-GGGCCGGACTGCTCATAAC-3'

### Western Blot Analysis

Proteins were extracted from HaCaT cells, then after 8 or 10% SDS-PAGE resolved, transferred to PVDF membranes (EMD Millipore). After blocking membranes using 5% nonfat dry milk, was incubated overnight with primary antibodies against Bax, Caspase 8, Caspase 9 (1:1000; Abmart); Caspase 3, Bcl-2, p-mTOR/mTOR, p-P38/P38,  $\beta$ -actin, p-P44/42/P44/42, and GAPDH (1:1000; CST); p-PI3K/PI3K (1:500; Abcam). We used HRP-conjugated goat anti-rabbit IgG or goat anti-mouse IgG (1:8000; Abmart Biological Reagents Co., Ltd.) as the secondary antibodies to label the antibodies above.

## Immunofluorescence (IF) Studies

HaCaT cells ( $2 \times 10^4$ /well) were inoculated into a 4-well glass plate and subjected to signal-direct IF staining of p-Akt/Akt (1:200), p-P38/P38 (1:200), and p-mTOR/mTOR (1:100), followed by incubation with Alexa Fluor 488-conjugated secondary antibodies (OriGene Technologies, Inc.). DAPI (Beyotime Biotechnology, Inc.) was used to counterstain nuclei. We used a laser scanning confocal microscope (IX-83 FV1000; Olympus Corporation) to check the staining section and save all images.

## Inflammatory Cytokines Assay by Luminex in Psoriatic-Like Skin Inflammation Mice

Blood samples were collected from Con, IMQ, and IMQ+MCEO group mice. Separated serum was stored at  $-80^\circ\text{C}$ . Bio-Plex Pro Mouse Cytokine 1 6plx EXP (17007935, Bio-rad systems) was used to analyze the concentrations of IL-1 $\beta$ , IFN- $\gamma$ , IL-10, IL-17A, and TNF- $\alpha$  in one sample at the same time, follow the manufacturer's instructions.

## Statistical Analysis

We analyzed all data using GraphPad Prism version 8.0 (GraphPad Software, Inc. CA, USA). All indicators are represented as the mean  $\pm$  standard deviation, Using a one-way ANOVA or a two-tailed unpaired Student's *t*-test to calculate the difference. P-values were adjusted for multiple comparisons using Bonferroni correction. Values with  $P < 0.05$  were defined as statistically significant.

## Results

### Analysis of Matricaria Chamomilla Essential Oil Composition

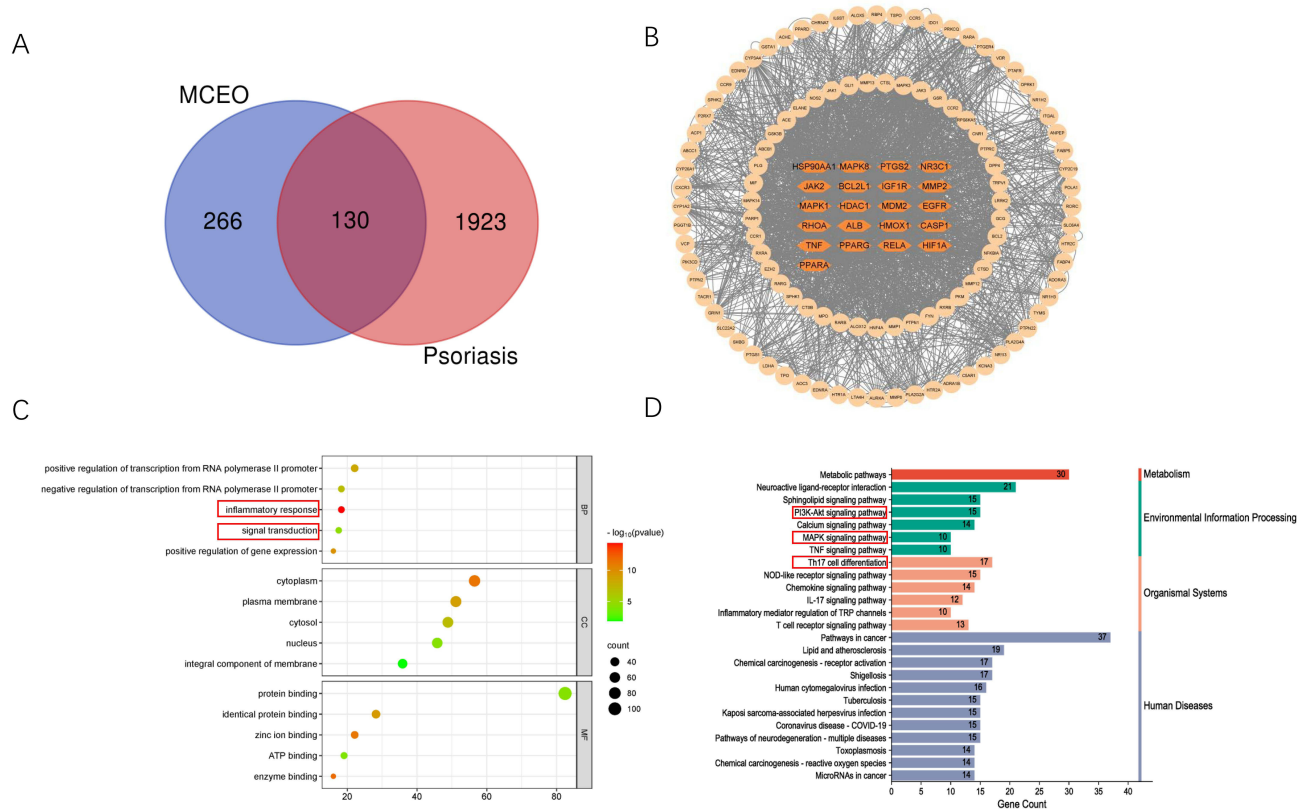
The components of *Matricaria chamomilla* essential oil were analyzed by GC-MS method, which mainly included 15 components (Table 1). Among them, Azulene accounted for the largest amount, about 88.9%.

### Analysis of Network Pharmacology

To explore the relationship between the active component of MCEO and psoriasis, the 12 components of MCEO were analyzed with PubChem. The intersection genes corresponding to the targets of MCEO and psoriasis were retrieved using the GeneCards database. The "Drug-target-disease" protein-protein interaction network (PPIN) was built using the STRING database. GO and KEGG enrichment analyses of common genes between MCEO and psoriasis were performed for further study. As shown in Figure 1A, 130 terms about MCEO and psoriasis were included, and PPIN was obtained

**Table 1** Composition of *Matricaria chamomilla* Essential Oil

Peak	Compounds	Peak Area (%)	Retention Time (Min)
1	Benzene,1,2,3,5-tetramethyl-	0.97	13.205
2	1,3,6-Octatriene, 3,7-dimethyl-	5.70	14.698
3	Bicyclo[4.1.0]hept-3-ene, 3,7,7-trimethyl-, (1S)-	0.7	14.964
4	Tricyclo[2.2.1.0(2,6)]heptane, 1,7,7-trimethyl-	0.93	15.402
5	Bisabolol	0.409	23.32
6	1,5,5-Trimethyl-6-methylene-cyclohexene	1.76	28.452
7	Isocaryophyllene	13.82	37.269
8	Cedrene	10.37	38.595
9	1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene-, [1ar-(1a.alpha.,4a.alpha.,7.beta.,7a.beta.,7b.alpha.)]-	29.16	40.181
10	Naphthalene, 1,2,3,5,6,8a-hexahydro-4, 7-dimethyl-1- (1-methylethyl)-, (1S-cis)-	17.95	42.909
11	(E,Z)-.alpha.-Farnesene	13.25	43.883
12	Azulene, 7-ethyl-1,4-dimethyl-	88.90	46.795
13	Cyclohexanol, 2-methyl-5-(1-methylethenyl)-, (1.alpha.,2.alpha.,5.beta.)-	15.23	47.585
14	n-Hexadecanoic acid	18.99	53.052
15	Dodecane	15.96	67.780



**Figure 1** Analysis of network pharmacology. **(A)** Venn diagram of intersection genes between MCEO and psoriasis; **(B)** PPIN was constructed with target genes between MCEO and psoriasis; **(C)** GO analysis of intersection genes between MCEO and psoriasis; **(D)** KEGG analysis of intersection genes between MCEO and psoriasis.

using a common gene between MCEO and psoriasis (Figure 1B). According to the GO analysis of molecular function (Figure 1C), the active targets are connected to inflammation response and signal transduction. KEGG analysis included the PI3K/Akt signaling pathway, MAPK signaling pathway, and Th17 cell differentiation (Figure 1D). These results guided further inflammation response and signaling pathway regulation assay.

## Analysis of Inflammatory Phenotype in Psoriatic Patients

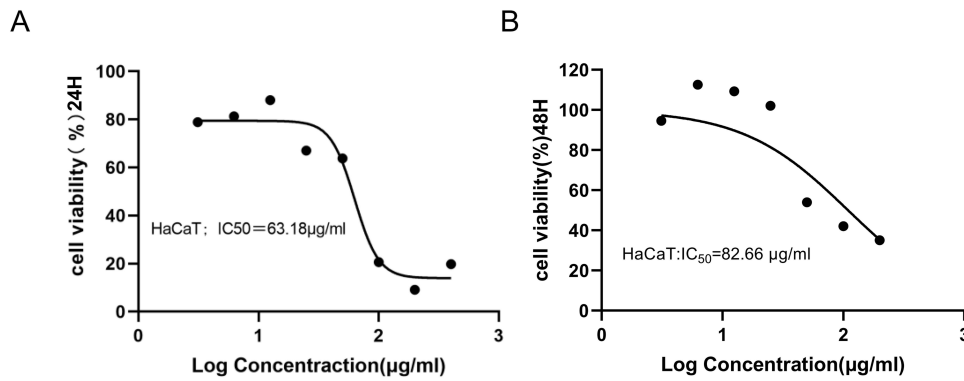
To clarify the expression characteristic of inflammatory cytokines in psoriatic patients, we collected the blood samples of 35 healthy and 31 psoriasis vulgaris patients and detected levels of IL-1 $\beta$ , TNF- $\alpha$ , IFN- $\gamma$ , IL-10, and IL-17A in the peripheral blood by Luminex (Supplement Table 1). Our results found that the levels of relative inflammatory factors appeared to increase in the peripheral blood of patients, except for IL-10 (Supplement Table 1), which grew gradually with the prolongation of the course of psoriasis, except for IL-10 (Supplement Figure 1).

## Drug-Regulated Verification in vitro Effect of MCEO on the Viability of HaCaT Cells

The viability of MCEO-treated HaCaT cells for 24h and 48h was tested using the CCK-8 assay. MCEO showed no obvious toxicity upon treatment, with concentrations ranging from 30–60  $\mu$ g/mL for 24h and 48h. A marked reduction in the activity of HaCaT cells was discovered upon treatment of cells with MCEO at concentrations above 63.18  $\mu$ g/mL for 24h and 82.66  $\mu$ g/mL for 48h (Figure 2A and B).

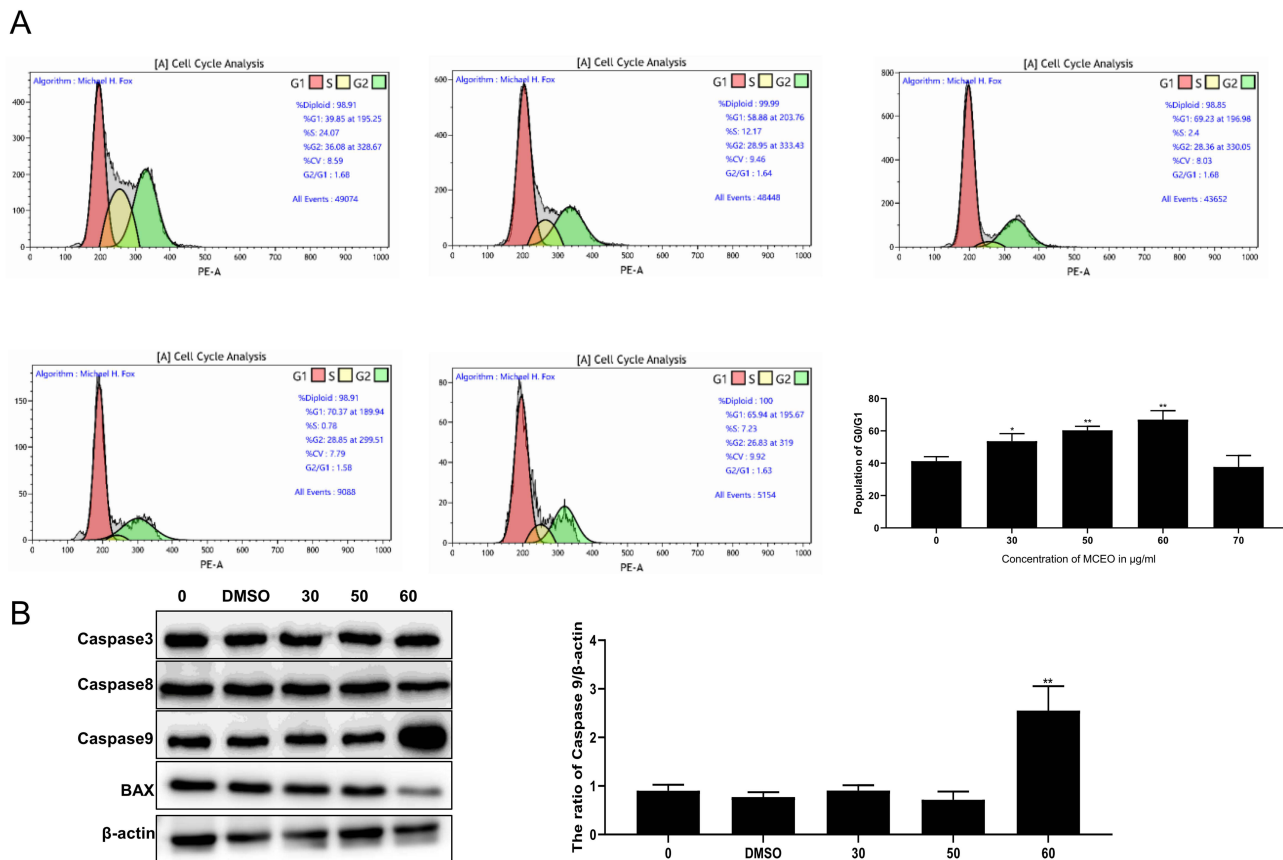
## Effect of MCEO on Cell Cycle and Apoptosis in HaCaT

To explore whether the effect of MCEO on cell viability was correlated with cell cycle arrest or apoptosis, we used flow cytometric analysis to measure the percentage of cells containing diploid (sub-G1) levels of DNA by PI staining. We used different concentrations of MCEO (30, 50, 60, and 70  $\mu$ g/mL) to treat HaCaT. Treatment with 50  $\mu$ g/mL and 60  $\mu$ g/



**Figure 2** Treatment with MCEO had no significant effect on the growth and viability of HaCaT keratinocytes. Relative numbers of viable HaCaT treated with or without MCEO (30–80 mg/mL) for 24 h (A) and 48 h (B) as determined by the CCK8 assay. Mean percentage viability of HaCaT plotted against the indicated doses of MCEO. Experiments were performed thrice with each concentration in triplicate wells, and the IC50 values calculated from these plots are shown. **Abbreviations:** MCEO, *Matricaria chamomilla* essential oil; NC, normal control; IC50, half-maximal inhibitory concentration.

mL of MCEO increased the proportion of HaCaT in the G1 phase (60.4±2.46% and 67.0±5.58%, respectively) compared with normal control (0 µg/mL) (41.3±2.7%)(*P*<0.01) (Figure 3A). Western blot (WB) analysis revealed that at a concentration of 60 µg/mL, MCEO treatment for 24h induced expression of caspase 9 apoptosis protein in HaCaT



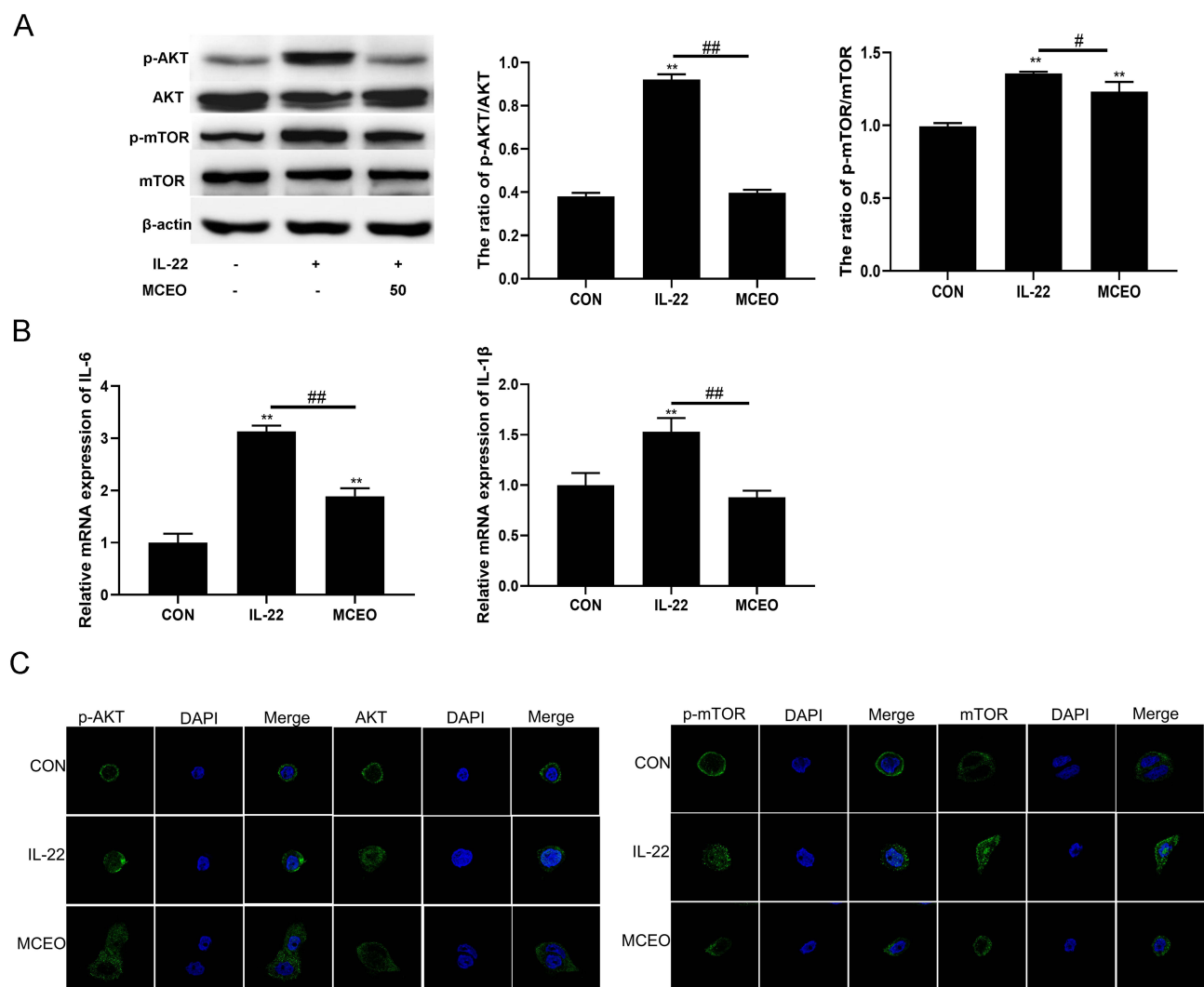
**Figure 3** Effects of MCEO on the percentage of cell population in different phases of cell cycle and the expression of apoptosis markers. (A) Histogram and column diagram show levels or percentages of distribution of MCEO-treated cells based on cell cycle, as assessed by flow cytometry analysis. (B) Expression levels of caspase 3, caspase 8, caspase 9, and Bax proteins, as assessed by Western blotting. Equal protein loading was confirmed using β-actin as the loading control. Bar graphs represent the mean ± SD of results obtained from three independent experiments performed in triplicate. \**P*<0.05 \*\**P*<0.01 vs.NC. **Abbreviations:** MCEO, *Matricaria chamomilla* essential oil; NC, normal control.



cells. At a 50 $\mu$ g/mL concentration, MCEO treatment for 24h did not induce apoptosis in HaCaT, as evidenced by the expression of Caspases 3, 8, and 9, and Bax protein levels (Figure 3B).

### MCEO Regulates IL-22-Induced HaCaT Proliferation and Inflammation by Inhibiting the PI3K/Akt/mTOR Pathway

In recent years, the immunological and pathological mechanisms have been clarified, which Th17 cells produce various cytokines, such as IL-17 and IL-22, which can induce proliferation, migration, or invasion of keratinocytes, release a potential ability in the pathogenesis of psoriasis.<sup>23–25</sup> IL-22 can also activate the Akt/mTOR pathway.<sup>26</sup> The PI3K/Akt/mTOR pathway up-regulates Th17 differentiation by promoting the nuclear translocation of ROR $\gamma$ t.<sup>27</sup> Hence, we measured the expression of IL-6, IL-1 $\beta$ , IL-17A, IL-10, and TGF- $\beta$  mRNAs by qRT-PCR (Figure 4B, some of the unchanged data were not shown). MCEO treatment decreased the levels of IL-6 and IL-1 $\beta$  mRNAs in HaCaT cells compared to those observed in the IL-22 treatment group, as shown by qRT-PCR ( $P < 0.01$ ;  $P < 0.01$ ). However, MCEO treatment did not change the expression of IL-17A mRNA in HaCaT. To clarify the anti-inflammation effect of MCEO exerted, the expression of PI3K, Akt, and mTOR proteins was detected by WB and IF in HaCaT treated with IL-22



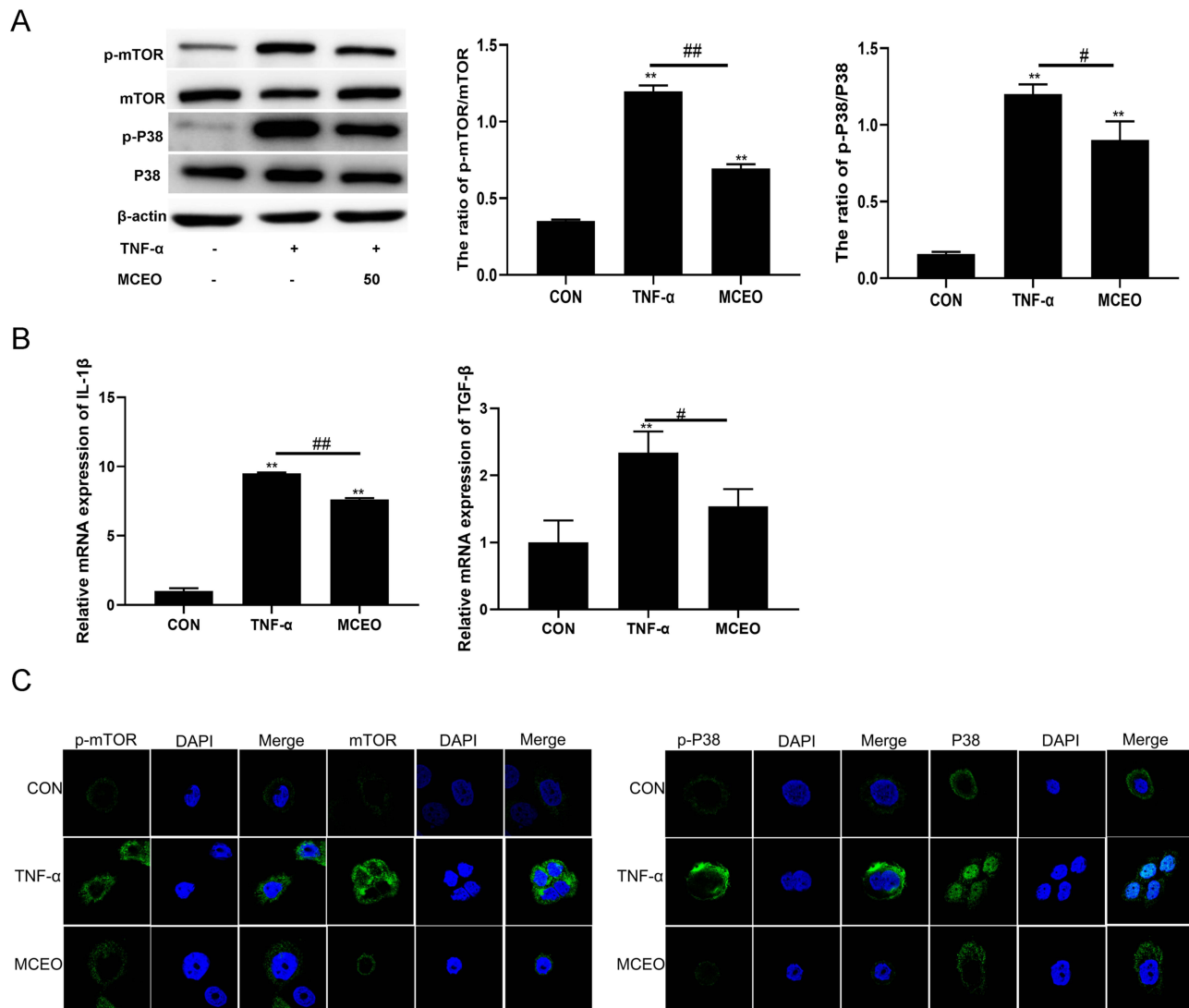
**Figure 4** MCEO regulates IL-22-induced HaCaT proliferation by inhibiting the PI3K/Akt/mTOR pathway. **(A)** HaCaT cells treated with or without IL-22 and MCEO were subjected to Western blotting using specific antibodies against p-Akt, Akt, p-mTOR, and mTOR. **(B)** The mRNA expression levels of IL-6 and IL-1 $\beta$  were analyzed by qRT-PCR. **(C)** HaCaT cells treated with or without IL-22 and MCEO were stained with p-Akt, Akt, p-mTOR, and mTOR and assessed using immunofluorescence. p-Akt, Akt, p-mTOR, and mTOR (green), nuclei (blue). Magnification,  $\times 2070$ . Bar graphs represent mean  $\pm$  SD of results derived from three independent experiments performed in triplicate. \*\* $P < 0.01$  vs NC; # $P < 0.05$  and ### $P < 0.01$  vs IL-22 group.

**Abbreviations:** MCEO, *Matricaria chamomilla* essential oil; NC, normal control; IL-22, interleukin-22.

(20ng/mL) for 24h after pretreatment with MCEO 24h (Figure 4A and C). Data showed that MCEO treatment significantly suppressed IL-22-mediated increase in the expression of p-Akt (at Ser473) and p-mTOR (at Ser2448) proteins compared with the control group, as assessed by WB and IF ( $P<0.05$ ;  $P<0.01$ ).

### MCEO Regulates TNF- $\alpha$ -Induced Inflammation by Inhibiting the PI3K/Akt/mTOR and p38MAPK Signaling Pathway

TNF- $\alpha$  is considered to be a key cytokine mediating skin inflammation during the development process of psoriasis.<sup>28</sup> It can accelerate lymphocyte, monocyte, and neutrophil infiltration, activated by dendritic cells and transferred from the peripheral blood into the skin.<sup>23</sup> This is related to the activation of Akt/mTOR and p38 MAPK pathways.<sup>26,29,30</sup> HaCaT cells were pretreated with 50 $\mu$ g/mL of MCEO for 24h before stimulation with 10ng/mL of rhTNF- $\alpha$ . We measured the expression of IL-6, IL-1 $\beta$ , IL-17A, IL-10, and TGF- $\beta$  mRNAs by qRT-PCR (Figure 5B, some of the unchanged data were not shown). Simultaneously, the activation of PI3K/Akt/mTOR and p38MAPK pathway was detected by WB and IF



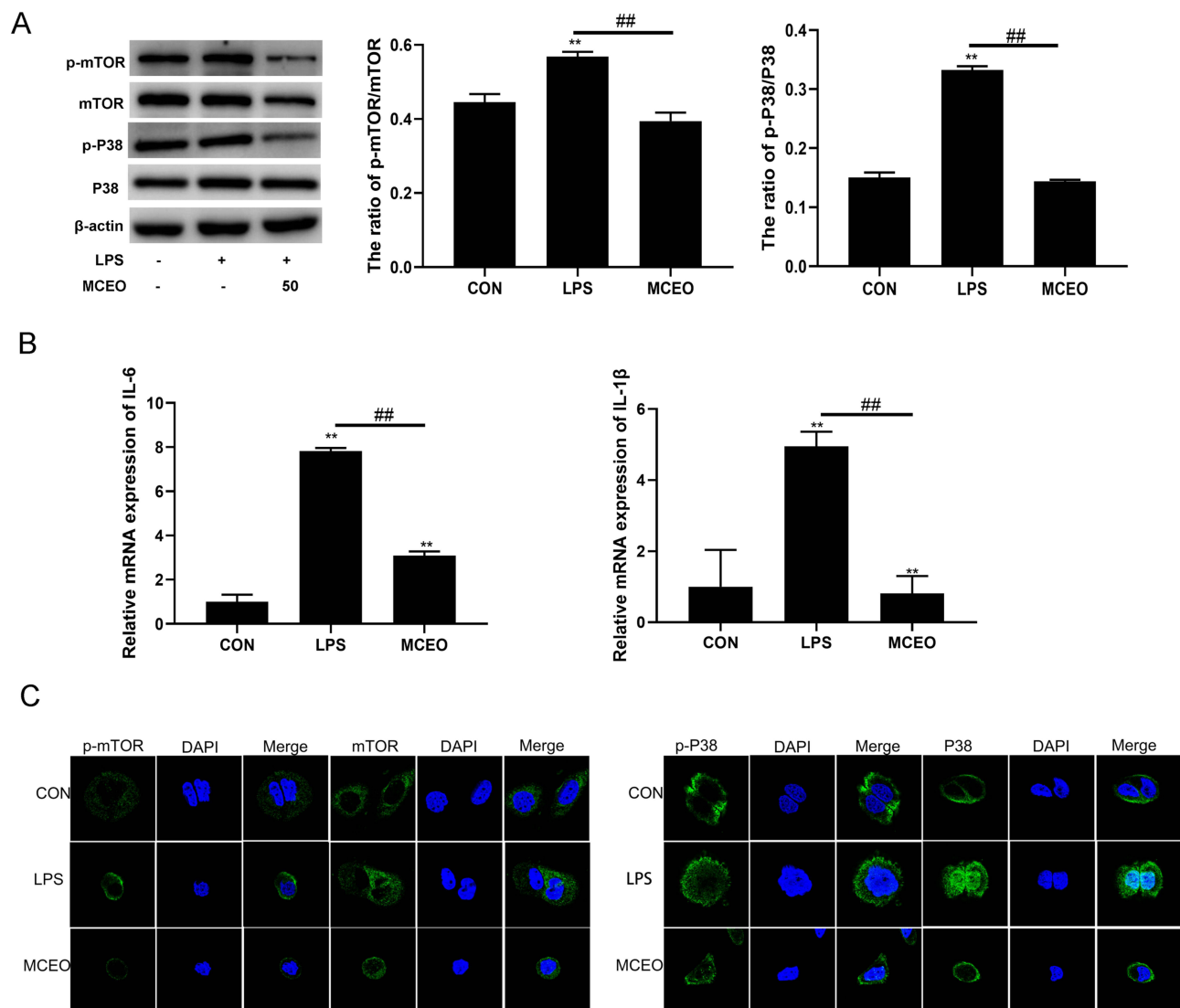
**Figure 5** MCEO regulates TNF- $\alpha$ -induced inflammation by inhibiting the PI3K/Akt/mTOR and p38MAPK signaling pathways. (A) HaCaT cells treated with or without TNF- $\alpha$  and MCEO were subjected to Western blotting using specific antibodies against p-P38, P38, p-mTOR, and mTOR. (B) The mRNA expression levels of IL-6, IL-1 $\beta$ , IL-17A, IL-10, and TGF- $\beta$  were analyzed by qRT-PCR. (C) HaCaT cells treated with or without TNF- $\alpha$  and MCEO were stained with p-Akt, Akt, p-mTOR and mTOR, and immunofluorescence was assessed. p-Akt, Akt, p-mTOR, mTOR (green), nuclei (blue). Magnification,  $\times 2070$ . Bar graphs represent mean  $\pm$  SD of results derived from three independent experiments performed in triplicate. \*\* $P<0.01$  vs NC; # $P<0.05$  and ### $P<0.01$  vs TNF- $\alpha$  group.

**Abbreviations:** MCEO, *Matricaria chamomilla* essential oil; NC, normal control; TNF- $\alpha$ , tumor necrosis factor- $\alpha$ .

(Figure 5A and C). Our results clarified that TNF- $\alpha$  treatment increased the expression of IL-1 $\beta$  and TGF- $\beta$  mRNAs and p-Akt, p-mTOR, and p-P38 proteins compared with the control group ( $P<0.05$ ;  $P<0.01$ ). However, pretreatment with MCEO for 24h markedly inhibited these TNF- $\alpha$ -mediated effects in HaCaT. The results clarified that MCEO possesses an anti-inflammation-specific impact, which is correlated with the downregulation of Akt/mTOR and p38MAPK pathways activation.

### MCEO Regulates LPS-Induced Inflammation by Inhibiting the PI3K/Akt/mTOR and p38MAPK Signaling Pathways

Cell cultures stimulated with LPS are routinely used to elucidate mechanisms of molecular immunity, and a model to assess the anti-inflammatory properties of new agents has been developed.<sup>31–33</sup> In our study, we used LPS (10 $\mu$ g/mL, 1h) to stimulate HaCaT cells pretreated with 50 $\mu$ g/mL of MCEO for 24h. We then detected the expression of IL-6, IL-1 $\beta$ , IL-17A, IL-10, and TGF- $\beta$  mRNAs by qRT-PCR (Figure 6B, some of the unchanged data were not shown). At the same



**Figure 6** MCEO regulates LPS-induced inflammation by inhibiting the PI3K/Akt/mTOR and p38MAPK signaling pathways. **(A)** HaCaT cells treated with or without LPS and MCEO were subjected to Western blotting using specific antibodies against p-Akt, Akt, p-P38, P38, p-mTOR, and mTOR. **(B)** mRNA expression levels of IL-6, IL-1 $\beta$ , IL-17A, IL-10, and TGF- $\beta$  were analyzed by qRT-PCR. **(C)** HaCaT cells treated with or without LPS and MCEO were stained with p-P38, P38, p-mTOR, and mTOR, and immunofluorescence was assessed; p-Akt, Akt, p-mTOR, mTOR (green), nuclei (blue). Magnification,  $\times 2070$ . Bar graphs represent mean  $\pm$  SD of results derived from three independent experiments performed in triplicate. \*\* $P<0.01$  vs NC; ### $P<0.01$  vs TNF- $\alpha$  group.

**Abbreviations:** MCEO, *Matricaria chamomilla* essential oil; NC, normal control; LPS, lipopolysaccharide.

time, the activity of PI3K/Akt/mTOR and p38MAPK pathways was monitored by WB and IF (Figure 6A and C). Our results clarified that LPS treatment increased IL-6 and IL-1 $\beta$  mRNA levels and p-Akt, p-mTOR, and p-P38 proteins compared to those observed with normal control ( $P < 0.01$ ). However, these LPS-induced effects were markedly suppressed upon pretreatment with MCEO for 24h. The results clarified that MCEO possesses an anti-inflammation-specific impact, which is correlated with the downregulation of Akt/mTOR and p38MAPK pathways activity.

## Drug-Regulated Verification in vivo

### MCEO Ameliorates IMQ-Induced Psoriatic-Like Skin Inflammation in Mice

To ascertain the function of MCEO on skin inflammation, we utilized an IMQ-induced psoriatic-like skin inflammation model in BALB/c mice, which were treated with or without MCEO (Figure 7A). Compared with the IMQ group, MCEO (16mg/time/mice) treatment alleviated the clinical symptoms of IMQ-induced psoriasiform dermatitis, including erythema, thickening, and scaling (Figure 7B). HE staining showed that MCEO treatment significantly ameliorated the histological changes mediated by IMQ compared to those observed in the IMQ group not treated with MCEO (Figure 7D). PASI scores in the IMQ+MCEO group were consistently lower than in the IMQ group (Figure 7C), while the IMQ group showed higher epidermal thickening than that observed in the IMQ+MCEO group (Figure 7E).

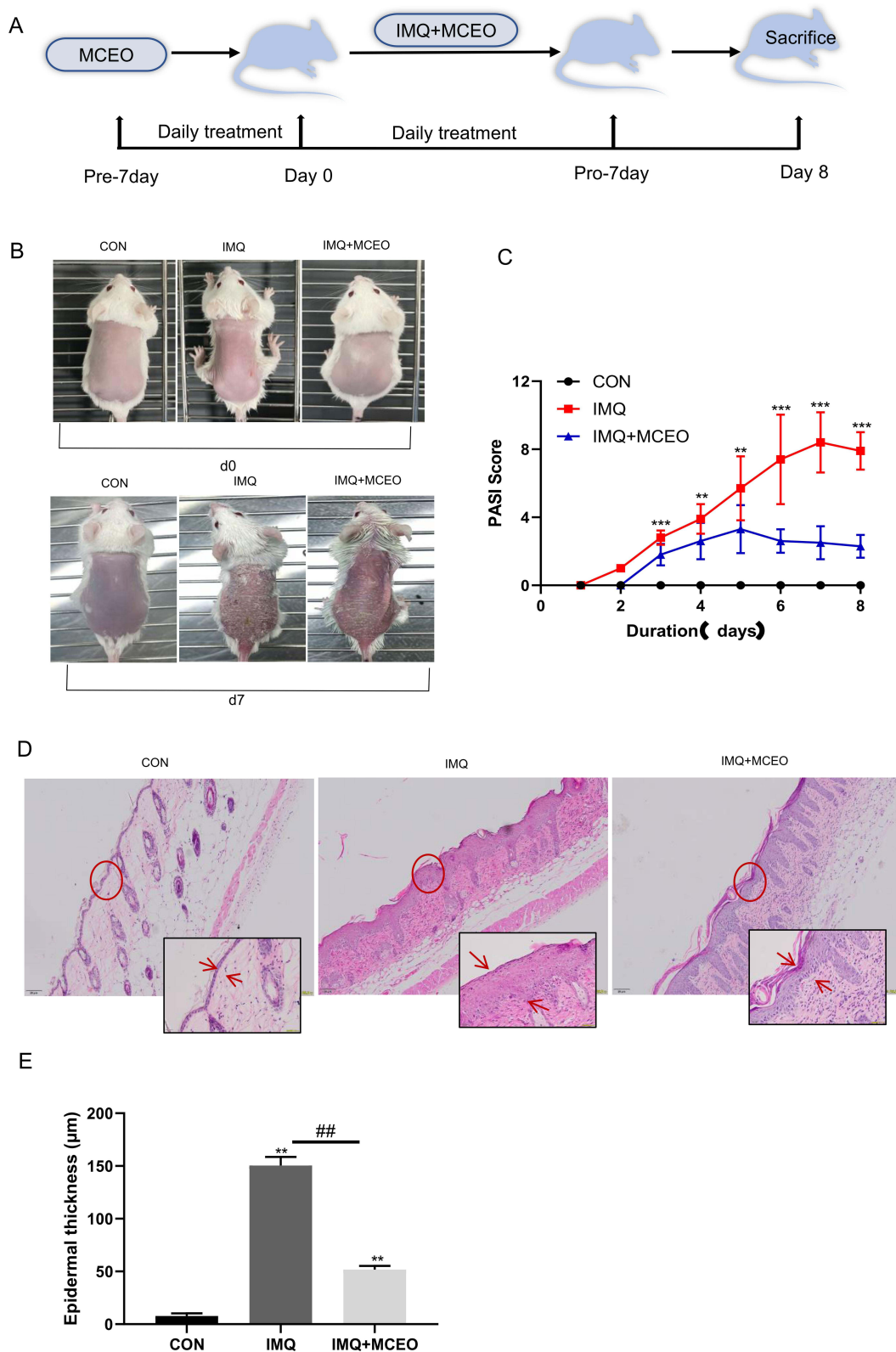
### MCEO Regulates IMQ-Induced Skin Inflammation by Inhibiting the PI3K/Akt/mTOR and p38MAPK Signaling Pathways in Mice

Comparative research clarified that activation of PI3K/Akt/mTOR and p38MAPK signaling pathways correlated with skin inflammation. In this study, we detected the activation of PI3K/Akt/mTOR and p38MAPK pathway was detected by WB (Figure 8A), and the levels of IL-6, IL-1 $\beta$ , TNF- $\alpha$ , IL-17A, IL-10, and TGF- $\beta$  mRNA was analyzed by qRT-PCR in the skin tissues of BALB/c mice (Figure 8B, some of the unchanged data were not shown). To further confirm the levels of inflammatory cytokines in IMQ-induced psoriatic-like dermatitis mice, IL-1 $\beta$ , TNF- $\alpha$ , IFN- $\gamma$ , IL-17A, and IL-10 were detected by Luminex in the serum of BALB/c mice (Figure 8C). Our data show that MCEO pretreatment downregulated the expressions of p-PI3K/PI3K, p-mTOR/mTOR, p-P38/P38, and p-ERK<sub>1/2</sub>/ERK<sub>1/2</sub> protein in skin inflammation mice. At the same time, we found that the expression of IL-6, IL-1 $\beta$ , TNF- $\alpha$ , IL-10, and TGF- $\beta$  mRNAs in skin tissue and levels of IL-1 $\beta$ , TNF- $\alpha$ , and IFN- $\gamma$  in serum were decreased by MCEO pretreatment. These results imply that MCEO ameliorates IMQ-induced psoriatic-like skin inflammation in mice, which is associated with inhibiting the activation of PI3K/Akt/mTOR and p38MAPK signaling pathways.

## Discussion

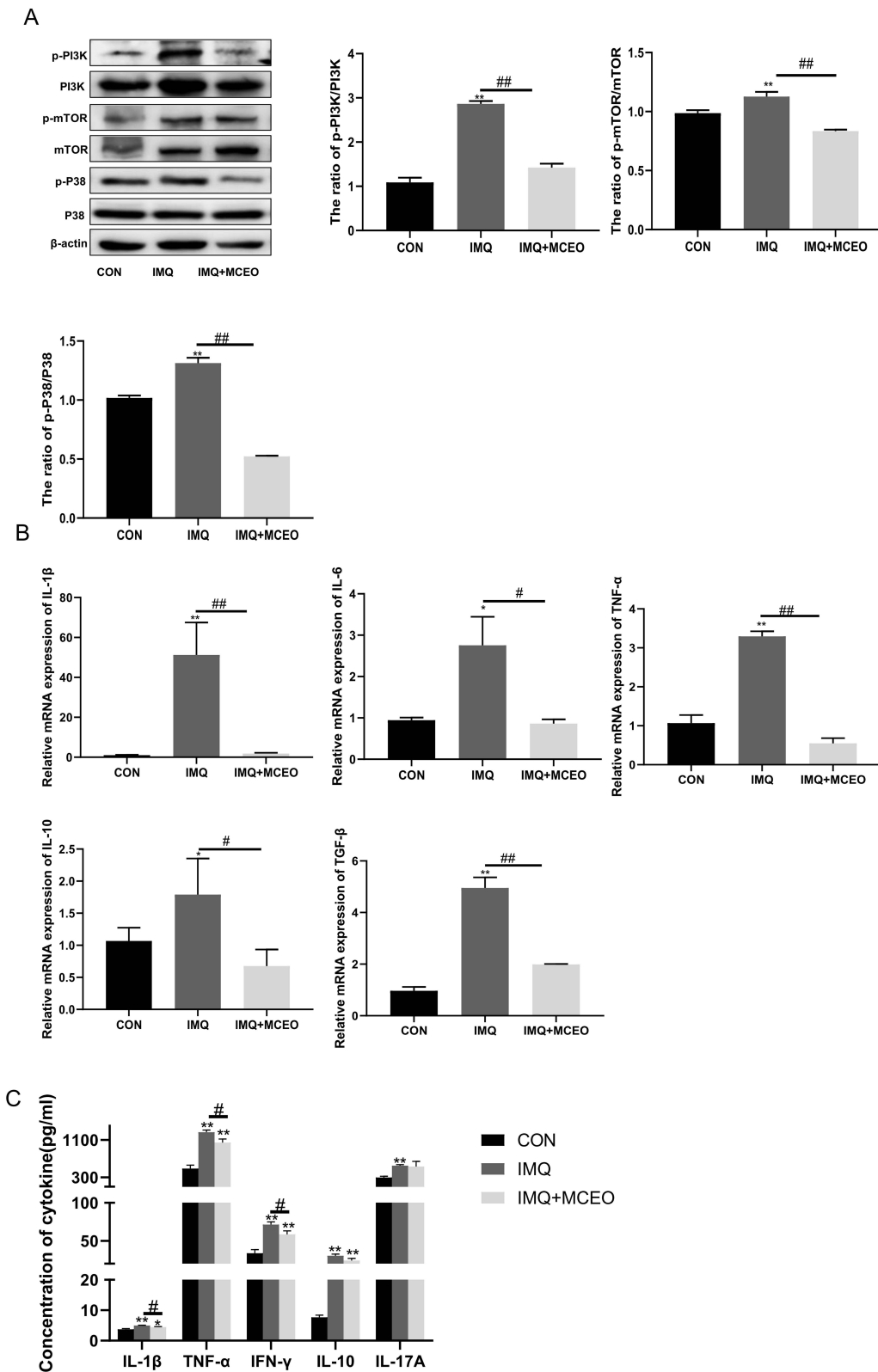
In this research, we first analyzed the components of MCEO by GC-MS; the intersection genes corresponding to the targets of MCEO and psoriasis were retrieved using the GeneCards database. GO and KEGG enrichment analyses of MCEO to psoriasis found that the active targets essentially connected to inflammation response and signal transduction, closely correlated with the PI3K/Akt signaling pathway, MAPK signaling pathway, and Th17 cell differentiation (Figure 1). These results guided further cytokines and pathway regulation assay. Following this, we evaluated the levels of relative inflammatory cytokines in psoriatic patients to define potential targets for the anti-inflammatory effects of MCEO in humans. We verified for the first time the anti-inflammation effect of MCEO in vitro and vivo experiments according to the predictive results of network pharmacology. Data showed that MCEO pretreatment inhibited inflammation of the HaCaT human keratinocytes induced by TNF- $\alpha$ , IL-22, and LPS, which was, at least partly, correlated with the regulation of PI3K/Akt/mTOR and p38MAPK pathway activity.

Moreover, MCEO exerted favorable effects for IMQ-induced psoriatic dermatitis in BALB/c mice, mainly reflected by the remission of erythema, thickening, induration, and scaling, and alleviating general skin injury. Even MCEO downregulated the levels of IL-1 $\beta$ , IL-6, TNF- $\alpha$ , and IL-10, but not IL-17A. These results indicated that MCEO has shown great value in preventing and treating psoriasis. In addition, MCEO, combined with different target anti-inflammatory plant drugs, could become a star in psoriatic skin inflammation treatment. Although the specific mechanism of action needs to be investigated further, MCEO may be an excellent agent for an anti-psoriatic-like skin inflammation drug.



**Figure 7** MCEO ameliorates psoriatic-like skin lesion in IMQ-induced mice. **(A)** Mice were pretreated with MCEO daily for seven consecutive days, followed by treatment with MCEO +IMQ daily for seven consecutive days and observation on day eight. **(B)** Photos of the dorsal skin of mice were captured on the eighth day after daily administration of IMQ+MCEO. **(C)** The cumulative PASI scores of all groups were also calculated daily. **(D)** H&E staining of the dorsal skin (magnification: 100 $\times$ , 400 $\times$ ), the distance between the red arrows represents the acanthoderm thickness and **(E)** epidermal thickness of the dorsal skin on day eight (scale bar: 100  $\mu$ m). Data are presented as mean  $\pm$  SD (n=6), \*\* $P$ <0.01 and \*\*\* $P$ <0.001 vs Psoriasis model group. ### $P$ <0.01 vs IMQ+MCEO group. One representative of three separate experiments is shown.

**Abbreviations:** MCEO, *Matricaria chamomilla* essential oil; NC, normal control.



**Figure 8** Mechanism of MCEO in ameliorating psoriatic-like skin inflammation in IMQ-induced mice. **(A)** The expression levels of p-PI3K/PI3K, p-mTOR/mTOR, and p-P38/P38 in the skin of mice with psoriasis were analyzed eight days after the treatment with MCEO by WB. **(B)** The mRNA expression levels of IL-6, IL-1β, IL-17A, IL-10, and TGF-β in the skin of mice with psoriasis were analyzed eight days after the treatment with MCEO by qRT-PCR. **(C)** The levels of IL-1β, IFN-γ, TNF-α, IL-10 and IL-17A in serum of mice with psoriasis were analyzed eight days after the treatment with MCEO by Luminex. Data are presented as mean ± SD (n=6), <sup>\*\*</sup>P<0.01 and <sup>\*</sup>P<0.05 vs Psoriasis model group. <sup>#</sup>P<0.05 and <sup>##</sup>P<0.01 vs IMQ+MCEO group. One representative of three separate experiments is shown.

**Abbreviations:** MCEO, *Matricaria chamomilla* essential oil; NC, normal control.

Psoriasis is an autoimmune disease; however, its exact pathogenesis remains unknown. Currently, synthetic drugs are commonly used to treat psoriasis, either by systemic injection or topical administration. However, the application of synthetic drugs shows many serious side effects in patients. As a result, novel, safer, and more effective natural botanical medicines have garnered the significant interest of researchers worldwide.<sup>34</sup> Plants and their secondary metabolites have a unique advantage in discovering potential anti-psoriasis agents.<sup>34,35</sup> Moreover, medicinal plants are easy to obtain and are cheap and effective. Treatment of psoriasis is reliant on them globally.<sup>36</sup> *Matricaria chamomilla*, a kind of ancient aromatic plant of the family Compositae, its base volatile oil, has shown potent effects in cosmetics, including eliminating skin inflammation and irritation and reducing skin redness.<sup>11,12,16</sup> Essential oils are a complex natural mixture of volatile secondary metabolites isolated from plants. The main components of essential oils are terpenes, and terpenes have been extensively studied for their anti-inflammation properties.<sup>37–39</sup> MCEO display a unique advantage in wound healing and skin barrier repair, which is related to their anti-inflammation, antioxidant, and antibacterial properties.<sup>10,16,40</sup> There are many kinds of ingredients in chamomile, such as cyclic ether, flavonoids, and total volatile oil, which can inhibit the growth of fungus. In addition, polysaccharides in the inflorescences have also shown significant anti-inflammatory effects, and MCEO also demonstrates anti-eczema effects.<sup>16,41–43</sup> Regrettably, the specific mechanism and functional active ingredients of MCEO in dermatology remain unelucidated.

Network pharmacology is a new strategy to systematically study the relationship between diseases, drugs, and targets.<sup>21</sup> MCEO include 16 components (Table 1). We obtained 130 MCEO network genes matched with the psoriasis gene set according to the results of GC-MS analysis. The PPIN network of MCEO was further analyzed with Cytoscape to explore psoriasis-related pathways. The network analysis using the MCEO compound network and psoriasis gene set demonstrated that the possible molecular mechanism of MCEO was highly associated with the PI3K/Akt, MAPK, and Th17 cell differentiation signaling pathway (Figure 1).

Inflammation is one of the important pathological features of psoriasis.<sup>44</sup> Our study used clinical patient samples to analyze inflammatory phenotype, including IL-1 $\beta$ , IFN- $\gamma$ , TNF- $\alpha$ , IL-10, and IL-17A. The levels of inflammatory cytokines increased gradually with the prolongation of the course of psoriasis, but not IL-10 (Supplement Table 1) (Supplement Figure 1). Cytokine IL-10 is a key anti-inflammatory mediator that protects the host from overreaction by pathogens and the microbiome, while playing an important role in other Settings such as sterile wound healing, autoimmunity, cancer, and homeostasis.<sup>45</sup> Maybe the downregulation of anti-inflammatory cytokines is also a cause of enhanced inflammatory response in psoriasis patients. These results indicated that inflammation is a determining factor during the progress of psoriasis. Therefore, anti-inflammatory therapy has become an effective entry point for exploring new therapeutic drugs.

Until now, no relative research has analyzed the effect of MCEO on inflammation in keratinocytes. First, we confirmed the optimal concentration of MCEO and analyzed its function on the viability and cell cycle of HaCaT cells by CCK8 and flow cytometry. Our data found that MCEO (50 $\mu$ g/mL and 60 $\mu$ g/mL) had no cytotoxic effect on HaCaT. Subsequently, we detected the expression of relative apoptotic proteins in HaCaT cells treated with MCEO (Figures 2 and 3). The expression of caspase 9 protein was obviously increased in HaCaT cells treated with MCEO (60 $\mu$ g/mL). We aimed to eliminate background effects to elucidate the mechanisms of MCEO underlying anti-inflammation effects. Hence, we selected 50 $\mu$ g/mL of MCEO for subsequent experiments.

We used IL-22, TNF- $\alpha$ , and LPS cytokines to stimulate HaCaT cells and establish an in vitro inflammatory keratinocyte model.<sup>46–48</sup> In this study, the mRNA expression of cytokines IL-1 $\beta$ , IL-6, TNF- $\alpha$ , IL-17A, TGF- $\beta$ , and IL-10 was analyzed by qRT-PCR. MCEO treatment significantly decreased IL-1 $\beta$ , IL-6, and TGF- $\beta$  mRNAs induced by IL-22, TNF- $\alpha$ , and LPS stimulation (Figures 4B, 5B, and 6B). In addition, IL-22 can activate the Akt/mTOR pathway,<sup>26</sup> TNF- $\alpha$  induces inflammation and the recruitment of immune cells, which is related to the activation of the MAPK/Akt pathway.<sup>49</sup> Liu et al found that LPS can act as a simulator, inducing inflammatory cytokines production in HaCaT cells, which is related to the induction of phosphorylation of p38MAPK.<sup>47</sup> p38MAPK participate in a broad spectrum of cellular responses induced by external stimulus. External stimuli include proinflammatory factors and stress, which mediates the release of IL-6, IL-1, IL-8, or TNF- $\alpha$  proinflammatory cytokines.<sup>50</sup> Consistently, our results clarified that MCEO treatment downregulated inflammation by controlling PI3K/Akt/mTOR and p38MAPK pathway activity and blocking the entry of p-mTOR and p-P38 into the nucleus in IL-22/TNF- $\alpha$ /LPS-induced HaCaT (Figures 4, 5 and 6).

Inflammation in dermatology is closely related to the PI3K/AKT/mTOR signaling axis, which is highly associated with the pathogenesis of skin disorders.<sup>51</sup> The mTOR pathway in pathogenic skin diseases perhaps participates in crosstalk with other signaling pathways directly or indirectly, such as MAPK, PTEN, AMPK, and PKC.<sup>51</sup> Consistently, we also found that MCEO cross-regulated the activation between PI3K/Akt/mTOR and p38MAPK pathway, which suppressed inflammation of HaCaT keratinocytes, perhaps via relative cytokines.

Since 2009, the mouse with IMQ-induced psoriatic-like skin inflammation has been widely used in preclinical psoriasis studies. Researchers usually use this animal model to study the efficacy, molecular mechanism, and absorption path of new drugs.<sup>52,53</sup> IMQ was smeared on the skin of mice to induce psoriasis-like symptoms. Many clinical symptoms were similar to psoriasis, including rash, scales, hyperplasia of the epidermis, and inflammatory cell infiltration in model mice were detected. MCEO pretreatment significantly ameliorated those symptoms (Figure 7A and B). The PASI scores and H&E staining of skin tissue showed that MCEO treatment relieved skin lesions of IMQ-induced BALB/c mice (Figure 7C and D). Moreover, MCEO treatment decreased the levels of IL-1 $\beta$ , TNF- $\alpha$ , IFN- $\gamma$ , and TGF- $\beta$ , but not IL-17A in mice with IMQ-induced skin inflammation, as assessed by qRT-PCR assay and Luminex, correlated with inhibiting PI3K/Akt/mTOR and p38MAPK pathway activity (Figure 8A and B). Therefore, we considered that MCEO could ameliorate skin inflammation in IMQ-induced psoriatic-like skin inflammation mice, which is involved in down-regulating the activity of PI3K/Akt/mTOR and p38MAPK pathway. Wang et al found that *German chamomile* volatile oil regulated T-cell lymphatic subpopulations to inhibit the Th17 cell differentiation signaling pathway, resulting in a reduction of interleukin 17 (IL-17), thereby inhibiting the activation of NF- $\kappa$ B and MAPK pathways, decreasing the secretion of the proinflammatory factors TNF- $\alpha$  and IL-6, and reducing inflammation.<sup>16</sup> In addition, extract besides the anti-inflammatory effect of *Matricaria chamomilla* extracts, we found MC aqueous extract effects as disrupting Th1/Th2 balance to Th1 upregulation.<sup>54</sup> Interesting, accumulated literature clarified that the IL-17/T-helper 17 (Th17) axis, a well-established proinflammatory cytokine, is involved in the pathophysiology of psoriasis.<sup>55,56</sup> We detected the level of IL-17A in clinical patient samples by Luminex and found that the secretion level of IL-17A increased gradatim with the prolongation of the disease course (Supplement Figure 1). We did not find the change tendency of IL-17A mRNA in HaCaT cells treated with TNF- $\alpha$ , IL-22, and LPS compared with normal control cells. However, in psoriatic-like skin inflammation model mice, we only observed that the level of IL-17A production in serum increased. MCEO treatment significantly reduced IL-1 $\beta$ , IFN- $\gamma$ , and TNF- $\alpha$  expression in the skin tissue and serum, but not IL-17A. This phenomenon indicates that IL-17A production is not associated with the activation of PI3K/Akt or MAPK signaling pathways. The way in which MCEO down-regulates inflammatory response does not affect IL-17A expression. There are reports that IL-17 inhibitors (ixekizumab and secukinumab) treated psoriasis as first-line clinical drugs. According to clinician experience and relative reports, targeting IL-17A obtained some surprising therapeutic effects in psoriasis.<sup>56</sup> Ho et al discovered that rosmarinic acid, a natural plant extract, is an IL-17A-targeting inhibitor that can block the IL-17A/IL-17RA axis to improve psoriatic skin inflammation in mice.<sup>57</sup> These new findings give us a new revelation: the combination of anti-inflammatory plant drugs with different targets can be selected, which may be a potential new approach for psoriasis therapy in the future field of psoriasis treatment.

To be mentioned, Azulene is one of the main components in MCEO, which has been recognized for its application in medicinal therapy against inflammation.<sup>58,59</sup> Azulene is one of these compounds whose anti-inflammatory potential have been shown by the previous studies, but a detailed analysis of its effect at the cellular level in terms of proinflammatory cytokine production has not been studied yet. In the following experiments, we will investigate the anti-inflammatory effect of Azulene on HaCaT cells in vitro and on IMQ-induced psoriatic-like skin inflammation.

## Conclusion

In the present study, we first discovered that MCEO, a plant oil, possesses a natural advantage in terms of skin barrier repair, which treatment inhibits inflammation in HaCaT keratinocytes, the potential mechanisms correlated with the activation and crosstalk of PI3K/Akt/mTOR and p38MAPK pathways. In addition, MCEO ameliorates the skin lesions in IMQ-induced psoriatic-like skin inflammation mice, downregulating the levels of inflammatory cytokines.

Finding novel, safe, and potential agents from natural plants for treating inflammatory skin disorders is a novel strategy for future research. Anti-inflammatory plant drugs with different targets with combined applications were



a potential therapeutic strategy in psoriasis. Our new findings provide a sufficient experimental and theoretical basis for the clinical application of MCEO.

## Ethics Approval and Consent to Participate

The human study was approved by the Ethics Review Committee on Human Research of Taizhou Second People's Hospital (tzey-ky202101). The animal studies were agreed upon by the Ethical Committees of Taizhou University (Taizhou, China) (TZXY-2022-20221027).

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## Disclosure

The authors have no conflicts of interest to declare for this work.

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