

Original Research

Active Ingredients of *Schisandra chinensis* Fruit Oil and their Effect on *Propionibacterium acnes*-Induced Inflammation in HaCaT Cells

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Abstract

Background: *Propionibacterium acnes* causes upregulation of inflammatory factors, such as cyclooxygenase-2, prostaglandin E2, interleukin-1 β , and tumor necrosis factor-alpha, increased levels of reactive oxygen species (ROS) and inward flow of calcium ions. This causes increased levels of the antimicrobial peptide LL-37 and inflammation of the skin, leading to redness, swelling, itching and other symptoms. *Schisandra chinensis* fruit oil (SCO) is rich in lignan active ingredients with various antioxidant and anti-inflammatory properties. **Methods:** In this study, SCO is obtained by supercritical CO₂ fluid extraction. SCO's anti-inflammatory actions were investigated using *P. acnes*-induced inflammation HaCaT cells model. A method based on reversed-phase high-pressure liquid chromatography with a diode array detector was developed and validated for the simultaneous detection of five lignan components. Levels of inflammatory factors and LL-37 were measured by ELISA kit and western blot respectively. Ca²⁺ and ROS levels detected by flow cytometry. **Results:** The experimental results show that the contents of schisanol A, schisanol B, schisanin A, schisanin B, and schisanin C were 33.89 \pm 0.24, 14.89 \pm 0.45, 8.92 \pm 0.02, 29.14 \pm 0.67, and 4.74 \pm 0.09 mg/g, respectively. Studies have demonstrated that SCO can alleviate skin inflammation by inhibiting the COX-2/PGE2 and NF- κ B signalling pathway. In addition, SCO can inhibit ROS production, significantly block inward Ca²⁺ flow, alleviate cell damage, and modulate the content of the antimicrobial peptide LL-37. **Conclusions:** In summary, our study elucidated the anti-inflammatory activity of SCO in a cell model and provided a scientific basis for its application as a raw material in skin care.

Keywords: *Schisandra chinensis* fruit oil; *propionibacterium acnes*; HaCaT cells; anti-inflammatory; NF- κ B signalling pathway; COX-2/PGE2 signalling pathway; LL-37

1. Introduction

Schisandra chinensis (Turcz.) Bail (SC) is a dried ripe fruit of the family Magnoliaceae [1]. It is a traditional Chinese herbal medicine with a long history of use. In traditional Chinese medicine, it is used to treat gastroenteritis, respiratory failure, cardiovascular disease, physical weakness, excessive sweating, insomnia, depression, anxiety, and many other disorders [1]. SC's main distribution sites are in the northeast and Inner Mongolia, Hebei, and Shanxi, China. Through literature research, it was found that SC has anti-inflammatory and antibacterial pharmacological effects [2–6]. Among them, lignans are the main potent constituents in SC, including schisanol A, schisanol B, schisanin A, schisanin B, and schisanin C [7]. The anti-inflammatory mechanism works by regulating the activity of inflammatory signalling pathways and reducing the production and release of inflammatory factors such as TNF- α , IL-1 β , and PGE2 [8–10]. SC also contains polysaccharides, terpenoids, organic acids, and flavonoids, among others [11–14].

Acne is a chronic inflammatory disease of the sebaceous glands of hair follicles [15]. The massive proliferation of *Propionibacterium acnes* in the pilosebaceous unit and chemical and cellular mediators that cause inflammation destroy the follicular sebaceous glands. As a result of their interaction, the skin microenvironment is altered, leading to an inflammatory response in the host and fostering acne lesion progression [16,17]. In addition to its direct antimicrobial action, LL-37 has a powerful immunomodulatory function and can act as an anti-inflammatory mediator with various immunomodulatory effects [18,19].

Cyclooxygenase-2 (COX-2) is undetectable in most tissues under physiological conditions but can be abundantly expressed in response to the induction of inflammatory factors [20,21]. COX-2 is the rate-limiting enzyme in prostaglandin E2 (PGE2) synthesis and is essential for regulating PGE2 activity [22,23]. PGE2 is an important inflammatory mediator that directly causes an increase in vascular tissue permeability and promotes the secretion of inflammatory factors such as interleukin (IL)-6 from rel-



evant tissues to induce an inflammatory response [24,25]. Activation of the COX-2/PGE2 pathway triggers skin pain, erythema, and pruritus.

NF- κ B is a central activator of several proinflammatory genes and plays a vital role in innate and adaptive immune cells and inflammation [26]. Activation of NF- κ B induces the production of proinflammatory mediators and molecules that lead to the inflammation, activation, and differentiation of immune cells [27]. Normally, the NF- κ B dimer binds to the inhibitor of NF- κ B (I κ B), preventing NF- κ B from translocating to the nucleus. When the cell is stimulated, the I κ B bound to the NF- κ B protein is phosphorylated and the phosphorylated I κ B is dissociated from NF- κ B and degraded by proteases, allowing NF- κ B to be released and quickly transferred from the cytoplasm to the nucleus. The nuclear NF- κ B binds to the promoter or enhancer κ B sequence in the target gene, thus acting as a nuclear transcript. NF- κ B leads to the release of mature IL-1 β and TNF- α [28,29]. Mature IL-1 β and TNF- α can stimulate the inflammatory response [28,30–32].

Many endogenous antimicrobial peptides (AMPs) have been shown to play essential roles in innate immunity [33,34]. The antibacterial protein LL-37 is the only antimicrobial peptide of the cathelicidin family expressed in humans [35,36]. It has significant antibacterial activity against both gram-negative and gram-positive bacteria. However, LL-37 is lacking in normal skin, but its secretion is significantly higher in inflamed skin than in non-inflamed skin [37,38]. One future trend is to consider antimicrobial peptides for treating skin inflammation [39]. LL-37 can inhibit the release of pro-inflammatory cytokines and promote the release of anti-inflammatory cytokines through the regulation of NF- κ B and other signalling pathways, thereby suppressing the inflammatory response and maintaining the immune homeostasis of the body [40].

Here, we used *P. acnes*-stimulated HaCaT cells to construct an *in vitro* inflammatory HaCaT cell model. This study investigated the effects of *S. chinensis* fruit oil (SCO), obtained by supercritical CO₂ fluid extraction, on inflammatory signaling pathways by determining the expression of inflammation-related factors and the antimicrobial peptide LL-37. In addition, this study aimed to demonstrate the effect of SCO on skin inflammation and validate its application in skin care.

2. Materials and Methods

2.1 Chemicals and Samples

HaCaT cells were purchased from Peking Union Medical College (Beijing, China), and Dulbecco's Modified Eagle's Medium (DMEM), dichloro-dihydro-fluorescein diacetate (DCFH-DA), and calcium fluorescent probe (Fluo-4, AM ester) were purchased from BIOAGRIO (Shanghai, China). The methyl thiazole tetrazolium (MTT) kit was purchased from Shanghai Biyuntian Biotechnology Co., Ltd (Shanghai, China), and trypsin, fetal bovine

serum (FBS), and a double antibody mix were purchased from Gibco (Grand Island, NY, USA). Phosphate-buffered saline (PBS) was obtained from HyClone (Logan, UT, USA). COX-2, PGE2, and IL-1 β ELISA kits were purchased from Shanghai WeiAo Biotechnology Co (Shanghai, China). The tumor necrosis factor- α (TNF- α) ELISA kit was purchased from Shanghai Enzyme-Linked Biotechnology Co (Shanghai, China). Acetonitrile (HPLC grade) was purchased from Shanghai Aladdin Biochemical Technology Co., Ltd (Shanghai, China), and standard controls of schisanol A (7432-28-2), schisanol B (58546-54-6), schisanin A (61281-38-7), schisanin B (61281-37-6), and schisanin C (61301-33-5) (purity >98%) were purchased from Shanghai Yuanye Biotechnology Co (Shanghai, China).

2.2 Supercritical CO₂ Fluid Extraction of SCO

The dried SC was crushed and passed through a 20 mesh sieve to obtain SC powder. SC powder (10.0 g) was accurately weighed and extracted using a Waters MV-10 ASFE supercritical fluid extraction system (Waters Corporation, Milford, MA, USA) at a CO₂ flow rate of 5 mL/min, extraction kettle temperature of 40 °C, extraction pressure of 200 bar, and an entrainer (ethanol or methanol) flow rate of 1 mL/min for 80 min. At the end of the extraction, ethanol was used as the rinsing solvent. The extract was collected, and the ethanol was removed by spin evaporation to obtain SCO.

2.3 Cyclooxygenase-2 Inhibition Determination

The inhibition of COX-2 by SCO at 0.50 mg/mL was measured according to the instructions of the COX-2 ELISA kit.

2.4 Cell Culture and Viability Assay

HaCaT cells were cultured in DMEM containing a mixture of 10.0% FBS and 1.0% double antibodies in an incubator at 37 °C and 5% CO₂. The HaCaT cells used have STR identification the results are 100% matched, and mycoplasma testing was negative.

HaCaT cells in the logarithmic growth phase were prepared at a cell concentration of 2×10^5 cells/mL, and 200 μ L of the cell suspension was added into each well of a 96-well plate and incubated for 12 h at 37 °C and 5% CO₂. Blank and sample groups were set up simultaneously, and the supernatant was discarded after incubation. Next, 20 μ L of MTT (0.5 mg/mL) was added to each well and incubated at 37 °C for 4 h. At the end of incubation, the supernatant was discarded, 150 μ L of DMSO was added to each well, and the absorbance value was measured at 490 nm after shaking for 30–60 min at 23.0 ± 2.0 °C. A safe concentration of the sample was determined when cell viability was greater than 80%. Cell viability was calculated as follows:

$$\text{Cell Viability\%} = \frac{\text{Sample } OD_{490}}{\text{Blank } OD_{490}} \times 100\% \quad (1)$$

2.5 Establishment and Validation of Cellular Models

HaCaT cells in the logarithmic growth phase were prepared at a concentration of 2×10^5 cells/mL, and 200 μ L of the cell suspension was added into each well of a 96-well plate and incubated for 12 h at 37 °C and 5% CO₂. Blank and model groups were set up; the blank group was not subjected to *P. acnes* stimulation, while 50 μ L of *P. acnes* at a concentration of 2.0×10^8 CFU/mL (OD_{600nm} = 0.5) was added to the model group for *P. acnes* stimulation (the cell survival rate of the model group was >80% by MTT assay). The 96-well plates were incubated at 37 °C with 5% CO₂ for 24 h, after which the supernatants were collected and assayed for PGE₂, IL-1 β , and TNF- α using ELISA kits according to the manufacturer's instructions.

2.6 Modulation of *P. acnes*-Induced Inflammatory Factors in HaCaT Cells by SCO

The safe concentration of SCO for HaCaT cells was measured using an MTT assay, and the appropriate concentration was screened for subsequent experiments based on the results of cytotoxicity experiments. The blank, model, and sample groups were set up separately; the blank and model groups were connected to 1.0 mL of culture medium, and the sample group was connected to 1.0 mL of sample diluted to a safe concentration with culture medium. Samples were incubated in a cell incubator for 6 h and then stimulated with *P. acnes*. *P. acnes* stimulation was added only to model and sample group, except blank. The cell supernatant was collected after incubation at 37 °C and 5% CO₂ for 18 h, and PGE₂, IL-1 β , and TNF- α were measured using ELISA kits according to the manufacturer's instructions.

2.7 RP-HPLC-DAD Assay

A method based on reversed-phase high-pressure liquid chromatography with a diode array detector (RP-HPLC-DAD) (1260 Infinity; Agilent Technologies, Santa Clara, CA, USA) was developed to determine the contents of five lignins simultaneously.

SCO was prepared at a concentration of 10.0 mg/mL in methanol, and the sample was separated on a reverse column ZORBAX SB-C18 (4.6 mm \times 250 mm, 5 μ m; Agilent Technologies). The mobile phases were 0.2% (v/v) acetic acid in water and acetonitrile (A and B, respectively), with an injection volume of 5.0 μ L, detection at 260 nm, and a column temperature of 30 °C. The liquid-phase elution gradients are presented in Table 1.

Table 1. Gradient elution method for SCO.

Time/min	A%	B%	Flow rate (mL/min)
0	55	45	1.0
10	35	65	1.0
30	35	65	1.0
35	25	75	1.0
40	25	75	1.0

2.8 Route and Mechanism of Action

To investigate the mechanism underlying the anti-inflammatory effect of SCO, Ca²⁺ and reactive oxygen species (ROS) levels were measured by flow cytometry (CytoFLEX, BECKMAN COULTER), and changes in I κ K α , NF- κ B, and LL-37 signaling pathways were investigated by western blotting.

2.8.1 Ca²⁺ Inward Flow

After *P. acnes* stimulation, the cells were incubated for 2 h and then washed thrice with PBS. Thereafter, 100 μ L of Fluo-4 AM was added to the cells and incubated for 20 min at 37 °C while protected from light. Next, Fluo-4 AM was removed, 200 μ L of trypsin was added to each well separately, and the cells were digested for 1–2 min before 200 μ L of the medium was added to terminate the digestion. Subsequently, the cytosol was removed, and 500 μ L of PBS was added to each tube to disperse the cells, after which Ca²⁺ was detected by flow cytometry.

2.8.2 Intracellular ROS Generation

After *P. acnes* stimulation and 2 h of cell culture, the cells were washed thrice with PBS. Afterward, 500 μ L of DCFH-DA working solution was added to each well and incubated for 30 min at 37 °C protected from light. After the same operation described in Section 2.5, PBS (500 μ L) was added to each tube, the cells were lysed, and ROS expression was detected by flow cytometry.

2.8.3 Western Blot Analysis

The same experimental procedure as that described in Section 2.5 was performed. After removal of the supernatant, the cytoplasmic protein extraction reagent was added to the collected cells to lyse them sufficiently and the cytoplasmic protein samples were harvested by centrifuging the supernatant. To the precipitate, cellular nucleoprotein extraction reagent was added and the supernatant was centrifuged to obtain a sample of cellular nucleoprotein. The concentration of bands on the membrane was analysed by gel preparation, loading, electrophoresis, transfer, blocking, incubation with antibodies and stain development to calculate the expression of I κ K α , NF- κ B and LL-37 proteins [41,42].

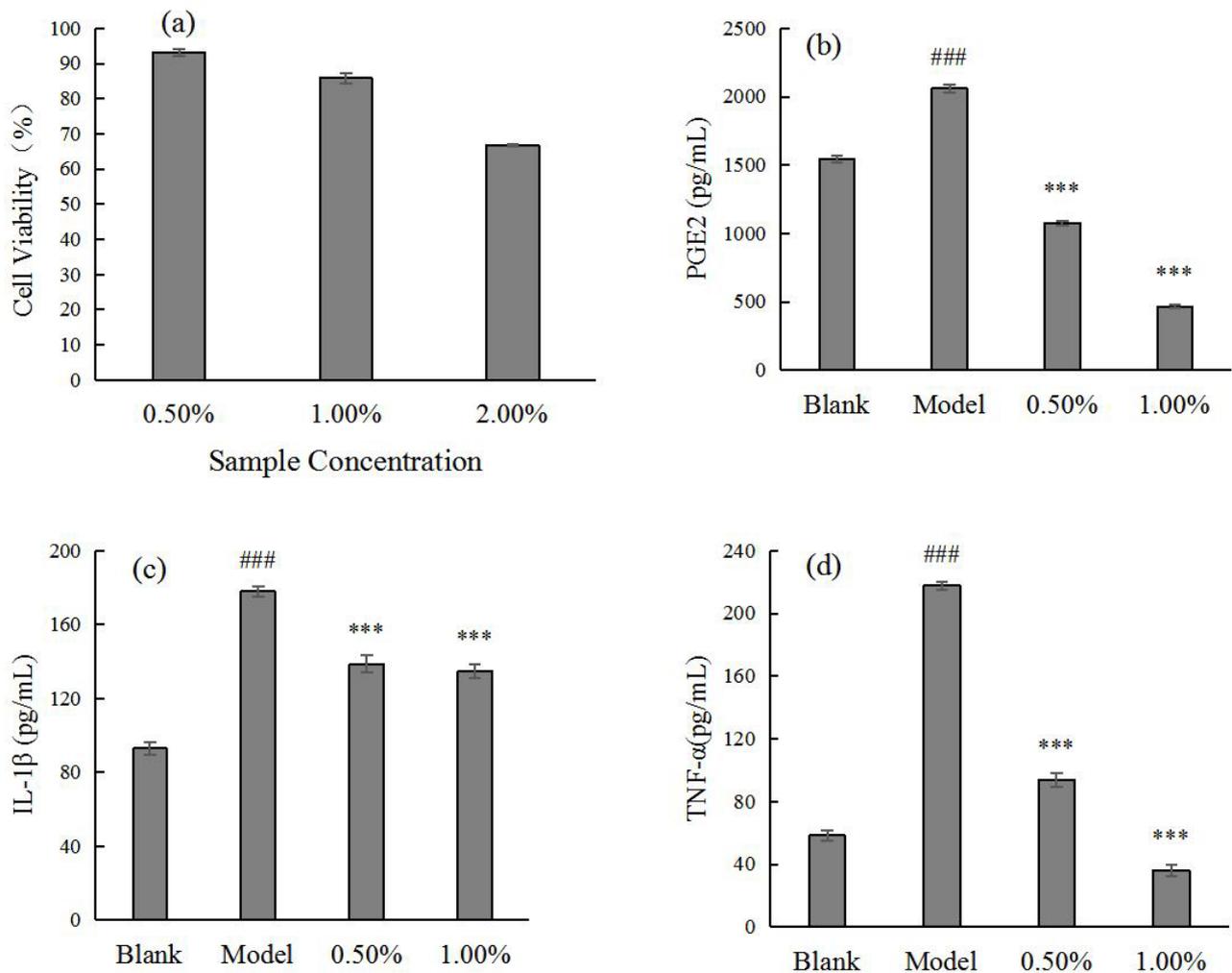


Fig. 1. SCO safe concentration for HaCaT (a) and the effect of SCO on PGE2 (b), IL-1 β (c), and TNF- α (d) expression in *P. acnes*-stimulated HaCaT cells. Data were analyzed via ANOVA. ### $p < 0.001$ compared with the blank; *** $p < 0.001$, compared with the model.

2.9 Statistical Analysis

All experiments were repeated thrice, and the results are expressed as mean \pm standard deviation (mean \pm SD). Statistical analysis was performed by applying SPSS 25.0 (SPSS Inc., Chicago, IL, USA) using one-way analysis of variance (ANOVA) plus a post hoc Duncan's test. ### $p < 0.001$ compared with the blank. ## $p < 0.01$ compared with the blank. *** $p < 0.001$, compared with the model. ** $p < 0.01$, compared with the model.

3. Results

3.1 Supercritical CO₂ Fluid Extraction of SCO

In the CO₂ supercritical fluid extraction process, the SCO yields obtained were $12.6 \pm 0.3\%$ and $18.0 \pm 0.5\%$ for methanol and ethanol as entraining agents, respectively. Therefore, ethanol was selected as an entraining agent to determine the anti-inflammatory efficacy of SCO.

3.2 Effect of SCO on COX-2 Inhibition

COX-2 is an inducible enzyme with cyclooxygenase and peroxidase activities and is a key enzyme in converting arachidonic acid to PGE2. COX-2 is expressed at very low levels under normal conditions but is significantly expressed in response to stimuli such as inflammation, pain, and tumors. Therefore, it can be used as an indicator of anti-inflammatory effects. The COX-2 inhibition experiments showed that SCO could inhibit COX-2 by up to 93.84% at 0.50 mg/mL, indicating that SCO has some anti-inflammatory potential.

3.3 SCO Reduces Levels of Inflammatory Factors

P. acnes causes skin inflammation, leading to the over-expression of inflammatory factors, such as PGE2, IL-1 β , and TNF- α , resulting in acne, redness, heat, erythema, and capillary dilation. As shown in Fig. 1, the levels of the inflammatory factors PGE2, IL-1 β , and TNF- α were signifi-

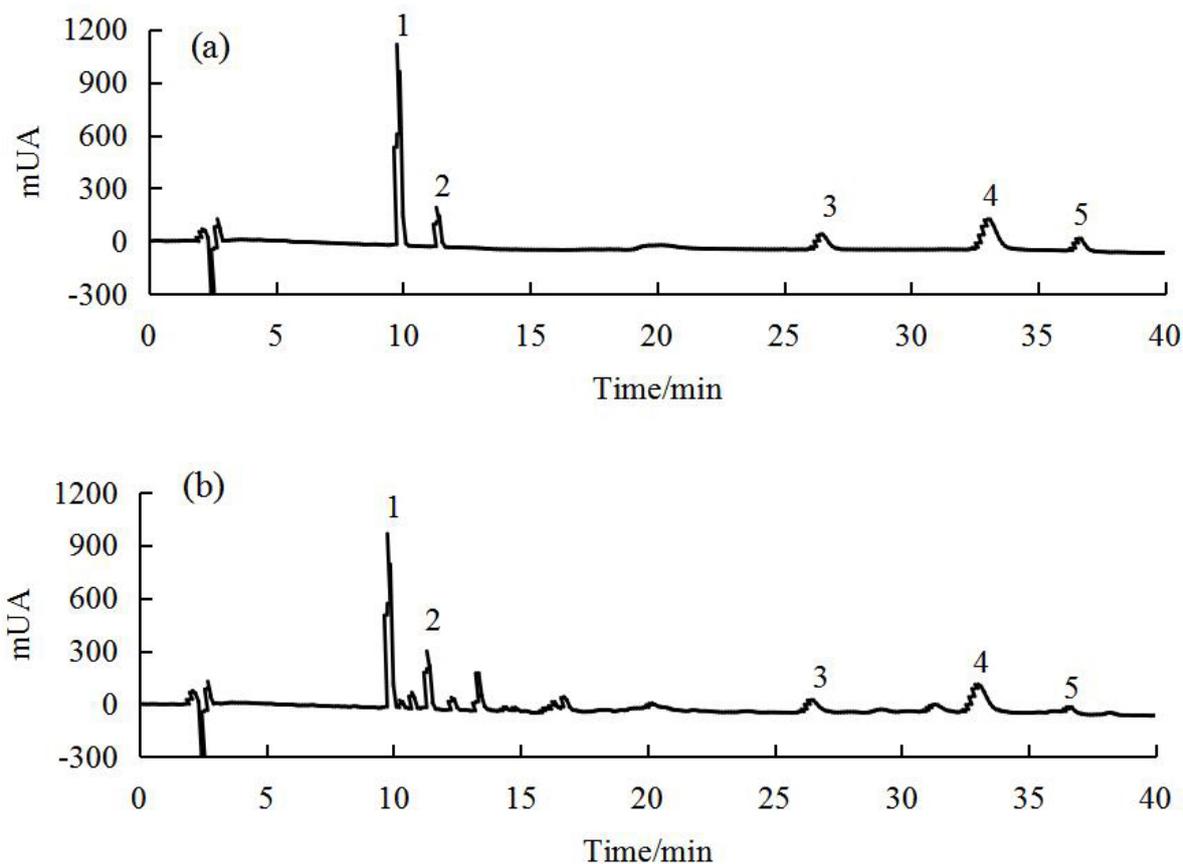


Fig. 2. RP-HPLC-DAD profiles of mixed standards (a) and SCO (b) (The numbers in the profiles: 1. schisanol A, 2. schisanol B, 3. schisanin A, 4. schisanin B, 5. schisanin C).

cantly increased in HaCaT cells after stimulation by *P. acnes* ($p < 0.001$), indicating that a HaCaT cell model of *P. acnes*-induced high expression of inflammatory factors was successfully established.

Thus, the effect of SCO on the expression levels of inflammatory factors induced by *P. acnes* in HaCaT cells was demonstrated using the above cellular model. The results showed that the cell survival rate could reach more than 80% at a concentration of 1.00% or less; therefore, a sample concentration of 1.00% or less was chosen for the experiment. The results in Fig. 1 show that SCO significantly reduced the expression of inflammatory factors PGE₂, IL-1 β , and TNF- α produced by HaCaT cells following stimulation by *P. acnes*, thus alleviating skin inflammation caused by *P. acnes*.

3.4 Active Constituents of SCO

Lignans as the main anti-inflammatory active component in SC [4,43,44]. The contents of schisanol A, schisanol B, schisanin A, schisanin B, and schisanin C are important indicators of SCO quality. Fig. 2 shows the RP-HPLC-DAD profiles of the mixed standards and SCO samples. The linear ranges and correlation coefficients (R^2) of the different standard curves are listed in Table 2.

The method was validated in terms of repeatability, precision, stability, and recovery. Five batches of samples were injected, and the relative standard deviation (RSD) values of each component were $<3.50\%$, indicating good repeatability between sample batches. The RSD of the peak area of each component was $<2.00\%$ for five consecutive injections of the same sample, indicating good instrument precision. The RSD of the peak area of each component was $<2.00\%$ for the same sample at 0, 6, 12, and 24 h indicating the good stability of the components within 24 h. The recoveries of the components were within 95.00–105.00% and RSD was $<3.00\%$ after three repetitions, indicating that the method was feasible.

The results showed that SCO comprises a wealth of lignin-like active ingredients, including schisanol A, schisanol B, schisanin A, schisanin B, and schisanin C at 33.89 ± 0.24 , 14.89 ± 0.45 , 8.92 ± 0.02 , 29.14 ± 0.67 , 4.74 ± 0.09 mg/g, respectively. A further illustration of the anti-inflammatory effects of SCO in terms of its composition [45].

3.5 The Effect of SCO on Ca²⁺ and ROS

When the skin is exposed to harmful stimuli, oxidants, such as ROS, are produced excessively, and the body's balance between resistance and promotion of oxidation is dis-

Table 2. Quantitative linear ranges and correlation coefficients (R^2) of the standards.

No.	Components	CAS#	Time/min	R^2	Linear range ($\mu\text{g/mL}$)
1	schisanol A	7432-28-2	9.841	0.9984	40–400
2	schisanol B	58546-54-6	11.385	0.9992	10–100
3	schisananin A	61281-38-7	26.495	0.9987	10–100
4	schisananin B	61281-37-6	33.055	0.9992	30–300
5	schisananin C	61301-33-5	36.643	0.9962	10–100

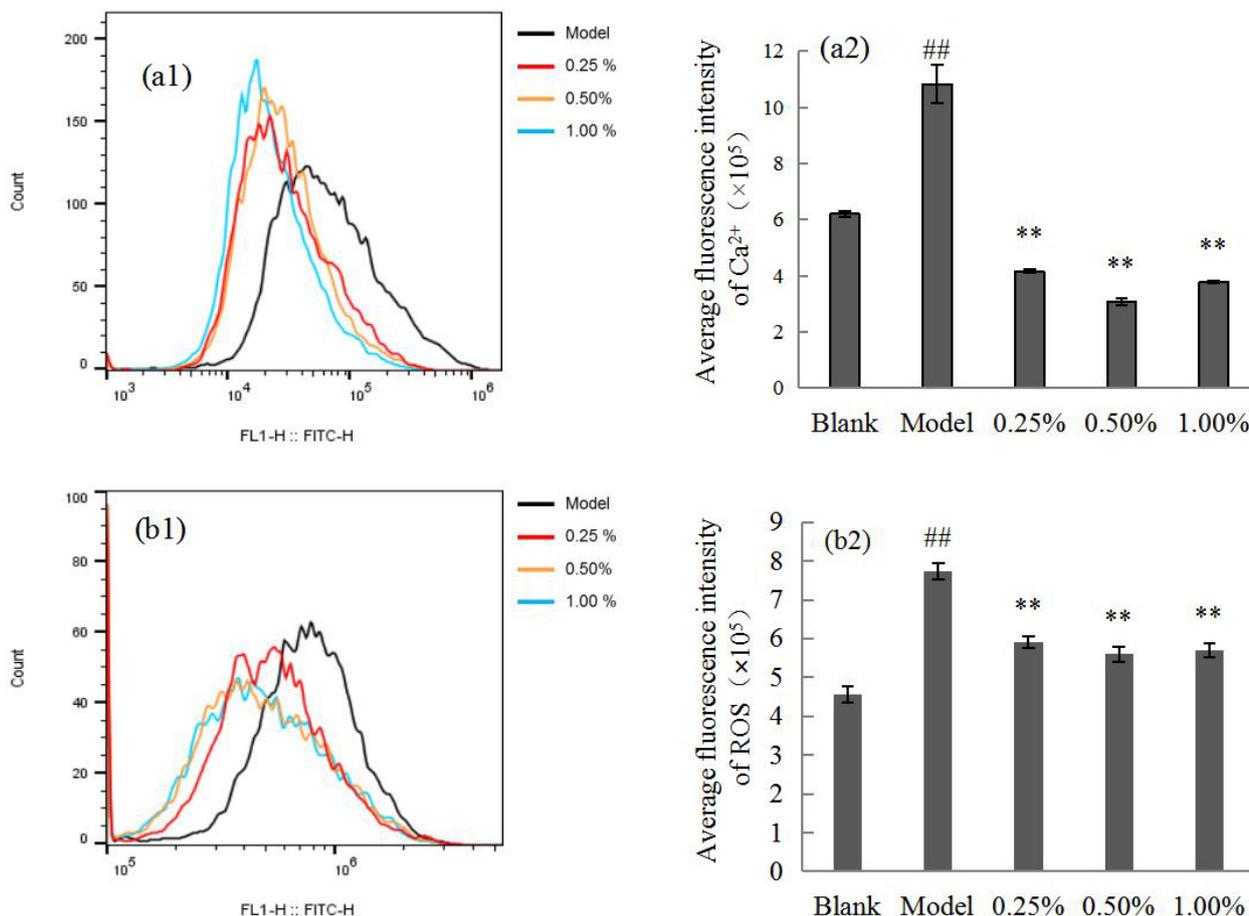


Fig. 3. Flow cytometry (a1,b1) showing the Ca^{2+} (a2) and ROS (b2) levels in HaCaT. ## $p < 0.01$ compared with the blank. ** $p < 0.01$, compared with the model.

turbed. Oxidative stress is generated by the excessive accumulation of free radicals in the body, which can also open Ca channels and promote inflammation and apoptosis [46,47].

Fig. 3 shows that Ca^{2+} and ROS levels were significantly higher in the model group compared with those in the blank control group ($p < 0.01$). Compared with that in the model group, SCO samples significantly ($p < 0.01$) reduced Ca^{2+} and ROS secretion levels at different concentrations, suggesting that SCO can significantly reduce Ca^{2+} and ROS levels, have significant antioxidant effects, inhibit downstream inflammatory pathways, and prevent skin damage caused by oxidative stress.

3.6 Effect of SCO on Changes in $\text{I}\kappa\text{K}\alpha$ and $\text{NF-}\kappa\text{B}$ Signaling Pathways

Quantitative analysis of western blot bands and grey scale values in Fig. 4 showed that SCO could significantly reduce the secretion of $\text{I}\kappa\text{K}\alpha$, indicating that SCO could inhibit the downstream production of inflammatory molecules by inhibiting the activation of $\text{I}\kappa\text{K}\alpha$. In addition, the relative expression of $\text{NF-}\kappa\text{B}$ in the nucleus and cytoplasm was measured, and the results showed that compared with that in the model group, SCO significantly inhibited the secretion of $\text{NF-}\kappa\text{B}$ in the cytoplasm and nucleus ($p < 0.01$). This indicates that SCO can inhibit the transfer of $\text{NF-}\kappa\text{B}$ from the cytoplasm to the nucleus after activation and thus inhibit the production of proinflammatory factors.

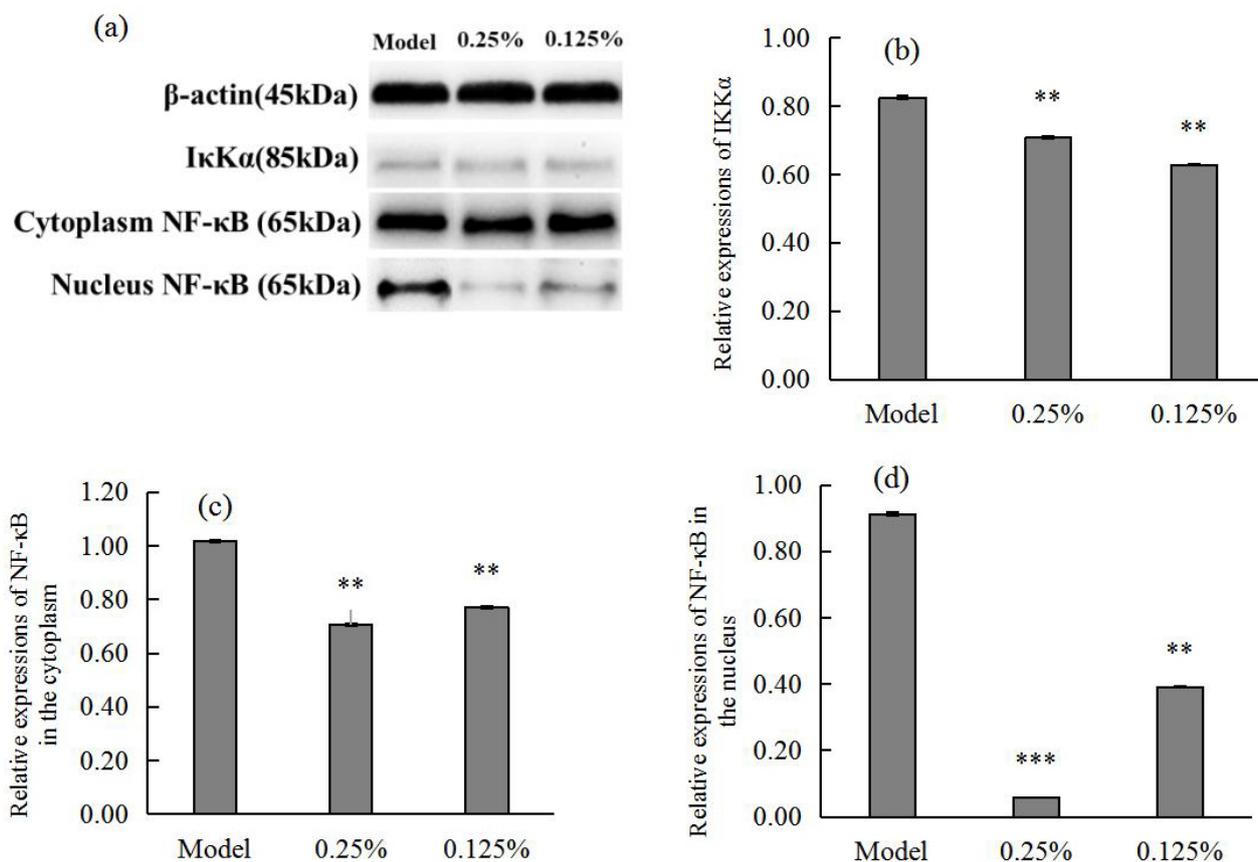


Fig. 4. Western blot images (a) showing IκKα (b) and NF-κB in the cytoplasm (c) and nucleus (d) in HaCaT cells. Data were analyzed via ANOVA. ** $p < 0.01$, compared with the model; *** $p < 0.001$, compared with the model.

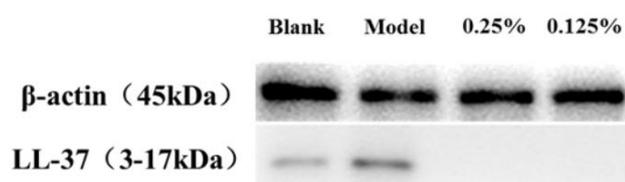


Fig. 5. Western blot showing the LL-37 levels in HaCaT.

3.7 Effect of SCO on Changes in LL-37

Fig. 5 shows that LL-37 secretion is significantly higher in the model group after induction, indicating that stimulation by *P. acnes* can activate the inflammatory response and promote the secretion of antimicrobial peptides. In contrast to that in the model group, SCO significantly inhibited the secretion of LL-37, indicating that SCO has a regulatory effect on antimicrobial peptides.

4. Discussion

This study aimed to determine the potential of SCO in regulating *P. acnes*-induced inflammatory factors in HaCaT cells. We focused on SCO, as it has been shown in various studies to have good anti-inflammatory and antiox-

idant properties, and its main active ingredients are lignans (schisanol A, schisanol B, schisanin A, schisanin B, and schisanin C). The lignans content of SCO was found to be 91.31 ± 1.47 mg/g. COX-2 is largely absent in normal physiological states and can be expressed in large amounts in the presence of inflammation. Therefore, preliminary evidence of the anti-inflammatory potential of SCO was obtained through COX-2 inhibition experiments *in vitro*.

As shown in Fig. 6, in the present study, we found that *P. acnes* causes increased levels of inflammatory factor secretion in HaCaT cells; therefore, we used this cellular model to assess the effect of SCO on inflammation and to understand the potential mechanisms by which SCO protects against inflammatory stress. IL-1β and TNF-α are important components for inducing the activation of downstream inflammatory responses and upregulating chemokines [48,49]. PGE2 expression can exacerbate peroxidative stress disorder by inducing local oxidative stress damage [24], and SCO downregulates the secretion of inflammatory factors, highlighting the anti-inflammatory potential of SCO. We further investigated the mechanism of SCO action. ROS production by *P. acnes*-stimulated HaCaT cells activates the IκB kinase (IκK) protein complex composed of IκKγ, IκKα, and IκKβ, followed by phos-

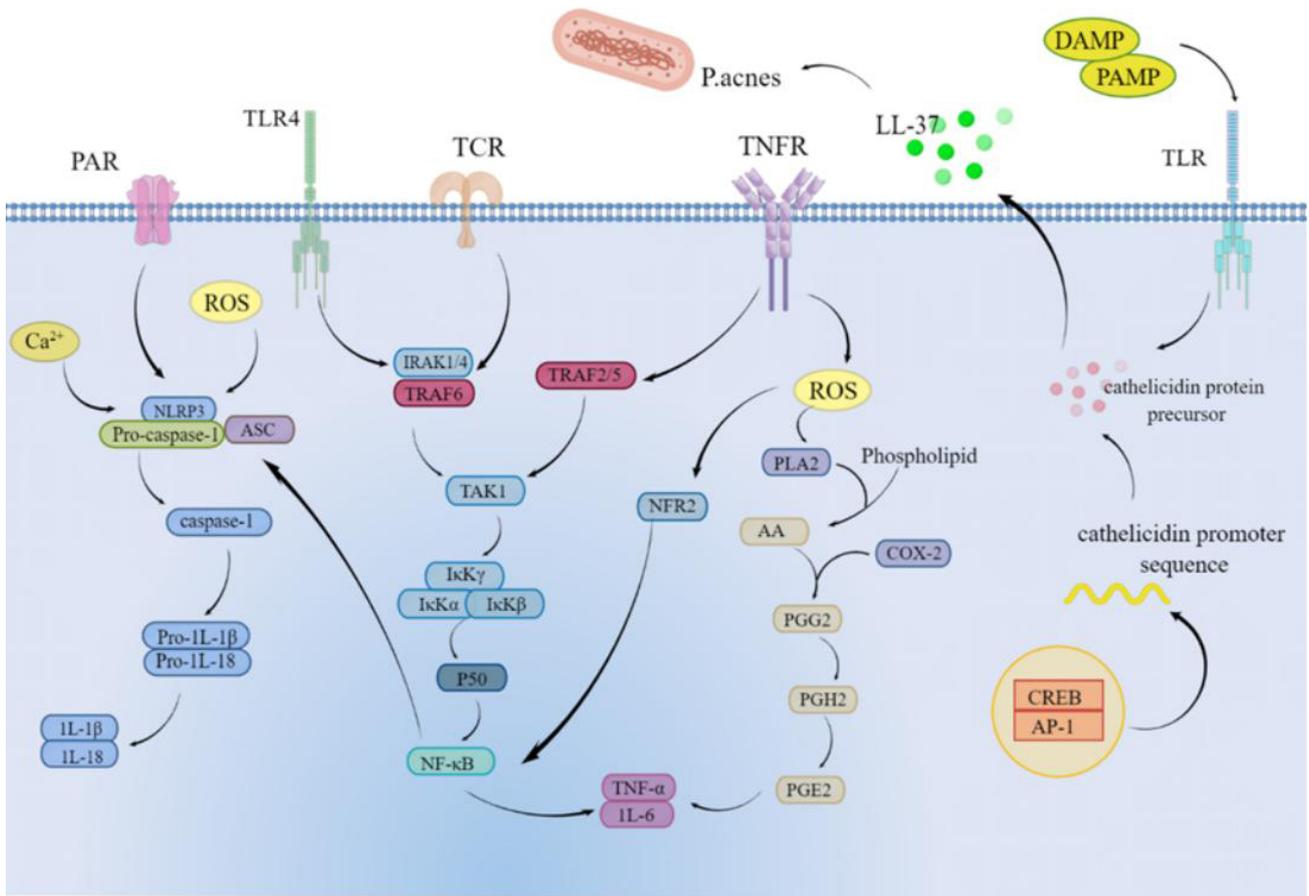


Fig. 6. Mechanism of action of SCO on *Propionibacterium acnes*-induced inflammation in HaCaT cells.

phorylation of I κ B protein to release NF- κ B, which can be transferred to the nucleus to regulate the expression of related genes [50,51]. In the present study, we found that SCO could inhibit the expression of ROS, effectively down-regulate the expression of I κ K α and NF- κ B, and inhibit the transfer of NF- κ B to the cell nucleus.

Ca²⁺ is a secondary messenger that mediates various biological responses in cells and plays an important role in maintaining normal cellular physiological functions [52]. The influx of Ca²⁺ into cells via Ca channels in the cell membrane leads to an overload of free Ca²⁺ in the cells, resulting in cellular damage [53]. The current study found that *P. acnes* caused significant Ca²⁺ inward flow, which was significantly inhibited by SCO, thereby alleviating cellular damage caused by *P. acnes* through Ca²⁺ inward flow.

LL-37 is the only cathelicidin AMP family expressed in humans and has been shown to play an important role in innate immunity [35,36]. LL-37 is lacking in normal skin, but its secretion is significantly higher in inflamed skin. Our results show that SCO can modulate LL-37 levels to alleviate skin inflammation.

The research results of Zhang *et al.* [3,6] showed that the extract of *Schisandra chinensis* and its active components played an anti-inflammatory role by acting on immune cells, reducing the secretion of pro-inflammatory cy-

tokines or acting on inflammation-related signaling pathways. Consistent with these results, this study suggests that *Schisandra chinensis* fruit oil can also play an anti-inflammatory role by reducing the secretion of pro-inflammatory cytokines and acting on the NF- κ B inflammatory signaling pathway, and regulate the secretion of LL-37. The structure-activity relationship between *Schisandra chinensis* fruit oil and its active ingredients should be further studied in order to provide certain basis for its application in food, medicine and skin care industries.

5. Conclusions

In this study, we found that SCO reduced the secretion of inflammatory factors and Ca²⁺ inward flow levels and decreased LL-37 levels through multiple pathways, thereby protecting HaCaT cells from the influence of *P. acnes* and alleviating the inflammatory reaction. Our study provides insights into the cosmetic and therapeutic applications of SCO in *P. acnes*-induced skin inflammation.

Availability of Data and Materials

Datasets used and/or analyzed for this study are available from the corresponding author upon appropriate request.

Author Contributions

LL, MT, YZ and MG designed the research study. HZ, HL and XM performed the research. PL, BC and DY provided research ideas and experimental guidance. YZ analyzed the data. HZ and HL wrote the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Ethics Approval and Consent to Participate

Not applicable.

Acknowledgment

Not applicable.

Funding

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Conflict of Interest

Author Pingping Lv, Meiling Tai, Biao Che was employed by the Infinitus (China) Co., Ltd. Author Dan Yu were employed by the company Beijing Lan Divine Technology Co.LTD. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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