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Original Article

Evaluation of antibacterial, anti-inflammatory, and anti-allergy properties of *Isodonis Japonicus* leaf/stalk extract

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Abstract

With societal advancements, improved living standards, and an increased focus on product safety, cosmetics with natural ingredients have gained substantial public interest. *Isodonis Japonicus* (*Isodon amethystoides* (Benth.) H. Hara), a traditional Chinese herbal medicine, exhibits a broad spectrum of pharmacological activities. The leaves and stalks of *Isodonis Japonicus* (XCC), effectively inhibit the proliferation of C. acnes, thereby contributing to the balance of skin microecology. QPCR and ELISA results indicate significant suppression of the secretion of inflammatory factors by XCC. Additionally, XCC mitigates free radical damage through the downregulation of ROS expression and exhibits soothing and anti-allergic properties by lowering mast cell counts, as well as IgE expression, pruritus, and ear swelling. These results indicate that XCC possesses remarkable antibacterial, anti-inflammatory, antioxidant, and anti-allergy properties. In conclusion, this study comprehensively investigates the pharmacological activities of XCC from multiple perspectives, elucidating its potential applications in skincare and the treatment of skin diseases.

Keywords: XCC extract, Antibacterial, Anti-inflammatory, Anti-allergy, Antioxidant

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Introduction

Natural products are invaluable resources, exhibiting a wide range of biological activities extensively utilized in medicine, healthcare products, cosmetics, and other fields (Foughalia et al., 2024; Tangthirasunun et al., 2024). The rise of synthetic cosmetics coincided with the rapid expansion of the petrochemical industry, leading to a preference for mineral oil due to its cost-effectiveness and high efficiency. However, since the 20th century, numerous cases of heavy metal poisoning and disfigurement from prolonged use of synthetic cosmetics have surfaced, resulting in significant societal repercussions (McMullen and Dell'Acqua, 2023). As societal attitudes and living standards have improved, there has been a noticeable shift towards cosmetics containing natural ingredients. Products formulated with natural plant components not only contribute to skincare but also exhibit minimal toxicity and side effects, ensuring enhanced safety (Kupradit et al., 2023).

The skin, recognized as the largest organ in the human body, consists of three primary layers: the epidermis, the dermis, and the subcutaneous tissues (Kumar et al., 2023). It performs several functions, including metabolic and homeostatic processes, as well as the excretion and absorption of substances, providing protection against various environmental aggressors such as pathogens, irritants, UV radiation, chemical threats, temperature fluctuations, and dehydration (Pérez-Sánchez et al., 2018). However, the accelerated pace of modern life has contributed to a multitude of skin-related issues, including inflammation, allergies, endocrine disorders, microbial infections, radiation damage, and aging effects (Liu, 2022). Neglecting proper skincare can cause minor health issues to develop into more severe pathological conditions, such as eczema, acne, rosacea, and seborrheic dermatitis. Thus, the importance of maintaining healthy skin has received significant attention.

Rabdosia nigrescens (Benth.) Hara., synonymous with *lsodon amethystoides* (Benth.) H. Hara., is a member of the labiaceae family and is extensively distributed throughout Northeast, North, and East China (www.worldfloraonline.org). Recognized under the International Nomenclature Cosmetic Ingredient (INCI) as *Isodon japonicus*, this species is identical to the aforementioned plants. Historically, it has been employed in traditional Chinese medicine for centuries. Comprehensive research indicates that *Isodon japonicus* is comprised of numerous chemical components, such as volatile oils, terpenes, flavonoids, steroids, etc., with a significant focus on terpenes. The active compounds derived from this species exhibit a broad spectrum of pharmacological functions, involving antimicrobial (Li et al., 2016), anti-inflammatory (Cummins et al., 2019), immunoregulatory (Guo et al., 2013), and antioxidative effects (Duan et al., 2021). Natural ingredients are highly valued in cosmetics and drugs due to their gentle and effective nature, alongside their safe and non-toxic characteristics. Furthermore, plant extracts consist of many bioactive compounds that demonstrate versatile properties applicable to both medicinal disorders and daily skincare routines (Michalak, 2023). In recent years, the increasing interest in natural products has directly impacted market demand for formulations containing these ingredients (Kurek-Górecka et al., 2020). Therefore, the development of plant-derived products for skincare is poised to meet consumer needs and align with market trends. Despite the known pharmacological properties of *Isodon japonicus*, its potential for use in topical skincare or treatment of skin diseases remains to be fully elucidated due to insufficient experimental data.

This study explored the potential applications of *Isodon japonicus* leaf/stalk extract, henceforth referred to as "XCC" (based on the company's internal designation), in skincare and skin diseases. The antibacterial efficacy of XCC against Cutibacterium acnes (C. acnes) was assessed using a minimum inhibitory concentration (MIC) assay. Moreover, its anti-inflammatory properties were evaluated through various inflammatory cell models induced by C. acnes, tumor necrosis factor (TNF)-α/interferon (IFN)-γ, and lipopolysaccharide (LPS). The anti-allergic effects of XCC were investigated using DNP-HAS-induced cell models and a calcipotriol-induced mouse model of allergic dermatitis. Flow cytometry was employed to assess the antioxidant effects of the extract. Through a comprehensive analysis of experimental results, the study elucidated the therapeutic value and prospects of XCC in skincare and skin diseases. The object of the study is to investigate the bioactivity of XCC, providing a scientific foundation for its prospective use in the cosmetics industry.

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Material and Methods

Chemicals and reagents

The dried leaf and stalk of *Isodon amethystoides* (XCC) were extracted with ethanol (70%) at 80℃ provided by Suzhou Pharmavan Co., Ltd. High-performance liquid chromatography (HPLC) was used for the quality control of XCC (Supplementary Fig. 1). All reagents mentioned herein are based on previously published reports (Gao et al., 2024).

C. acnes culture and preparation

C. acnes (ATCC 6919) was obtained from Zhili Zhongte Biotechnology Co., Ltd (Wuhan, China). The bacteria were cultured in BHI broth for 3-4 days under anaerobic conditions using the AnaeroPack system (MGC, Japan) at 37℃. A microplate reader measured the bacterial growth until an OD_{600} of 1.0 was reached. Subsequently, C. acnes were collected, washed, and subjected to heat treatment at 85℃ for 30 min to induce heat killing. The suspension of heat-killed C. acnes was stored at -70℃ until further use.

MIC determination

C. acnes were grown to logarithmic phase, pelleted, and washed twice with BHI broth. The bacteria were then resuspended at 5×10^4 CFU per well. Two-fold serial dilutions of XCC and salicylic acid in BHI broth were prepared and 100 μL was added to each corresponding well. After incubation in AnaeroPack at 37℃ for 48 h, the plate was taken out and observed in the light. The MIC was determined by the absence of bacterial growth in the wells relative to the controls. The inhibition rate was taken at 600 nm using a microplate reader.

Cell culture

Human peripheral blood lymphocytes and monocytes (PBMCs) were acquired from Milestone Biotechnology Co., Ltd (Shanghai, China). Following the manufacturer's instructions, the cells were thawed and then resuspended in RPMI 1640 medium, enriched with 10% (v/v) heat-inactivated FBS, and maintained at 37℃ in a 5% CO_2 atmosphere. Bone marrow cells (BMMCs), HaCaT, Raw264.7, and RBL-2H3 cells were isolated and/or cultured following previously described methods (Gao et al., 2024).

Real-Time Quantitative PCR

HaCaT and Raw264.7 cells were placed in 12-well plates at 2×10^5 cells/well. Following a 24-hour incubation, the cells were pretreated with XCC at the different concentrations of 0.2, 0.4, and 0.8 μg/mL for 1 h, followed by exposure to various stimuli for an additional 24 h. The stimulation conditions included heat-inactivated C. acnes at an MOI of 100 for both cell types. HaCaT cells were treated with IFN-γ and TNF-α at concentrations of 10 ng/mL, while Raw264.7 cells and PBMCs received LPS at 1 μg/mL. Relative mRNA expression levels were evaluated by calculating $2^{-\Delta\Delta CT}$ method. The sequences of the primers utilized are listed in Table 1.

Table 1. Primer sequences utilized for qRT-PCR.

Gene	Forward primers (5'-3')	Reverse primers (5'-3')
hIL-6	TCTGGATTCAATGAGGAGACTTG	TGGGTCAGGGGTGGTTATT
hIL-8	GCTCTGTGTGAAGGTGCAGTT	TTTCTGTGTTGGCGCAGTGT
hβ-actin	TGACGTGGACATCCGCAAAG	CTGGAAGGTGGACAGCGAGG
mIL-1β	AAAAAGCCTCGTGCTGTCGG	CAAGGCCACAGGTATTTTGTCG
miNOS	GAGACAGGGAAGTCTGAAGCAC	CCAGCAGTAGTTGCTCCTCTTC
mβ-actin	GAGACCTTCAACACCCCAGC	GAGACCTTCAACACCCCAGC

ELISA

Raw264.7 cells, RBL-2H3 cells, BMMCs, and human PBMCs were prepared according to the protocol described in "Real-Time Quantitative PCR". The supernatants from the cell cultures were collected. The tests were performed according to the manufacturer's guidelines.

Nitrite determination

Raw264.7 cells were cultured following the procedure outlined in "Real-Time Quantitative PCR". Nitric oxide (NO) production was assessed using the Griess Reaction Kit, with all steps conducted by the manufacturer's instructions.

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Measurement of mast cell degranulation

Cell processing and assay protocols have been described previously (Gao et al., 2024).

Flow cytometric analysis

HaCaT cells were placed in a 12-well plate and incubated for 24 h. The cells were treated with XCC at the different concentrations of 0.2, 0.4, and 0.8 μg/mL for 1 h and then exposed to 1000 μM H_2O_2 for 12 h. Following this, the cells were stained with 10 μM DCFH-DA in a serum-free medium and incubated at 37°C for 20 min before harvesting. Reactive oxygen species (ROS) production was analyzed via flow cytometry.

Animal experiments

This study received approval from the Institutional Animal Care and Use Committee at Suzhou Pharmavan Co., Ltd, Jiangsu Province, China (IACUC-YF02-21100901.01). Female BALB/c mice (18-20g) were sourced from SPF (Beijing) Biotechnology Co., Ltd and randomly divided into five groups (n = 10 each), including a control group (normal), a model group (MC903), and three treatment groups that received 0.01% and 0.1% of XCC, as well as a TAC group (0.1% tacrolimus). A 2 nM MC903 solution in 20 μL of 100% ethanol was administered repeatedly to the right ear. Starting from day 3, either TAC or XCC, in a consistent volume of 50 μL, was applied daily to the right ear for 8 days. The normal control group did not receive any treatment. On day 10, mice were individually in separate cages and video-recorded for 15 min immediately post-modeling. The number of scratching behaviors for each mouse was subsequently counted. On day 11, after measuring ear thickness, the mice were euthanized and blood samples, along with ear tissues, were collected for IgE detection and toluidine blue staining.

Statistical analysis

A one-way ANOVA and Student's t-test were used to assess differences in both multiple and single comparisons, utilizing GraphPad Prism 8.0 (La Jolla, CA, USA). $P < 0.05$ was considered statistically. Data are presented as mean ± S.E.M. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ compared to the untreated group, and $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ compared to the stimulated group without XCC treatment.

Results

Effects of XCC on C. acnes growth

A MIC test was conducted to evaluate the antibacterial efficacy of XCC against C. acnes. XCC effectively inhibited the proliferation of C. acnes, exhibiting a MIC value of 2 μg/mL. In contrast, salicylic acid demonstrated a significantly weaker inhibitory effect on C. acnes, with an MIC exceeding 1000 μg/mL (Fig. 1a and 1b).

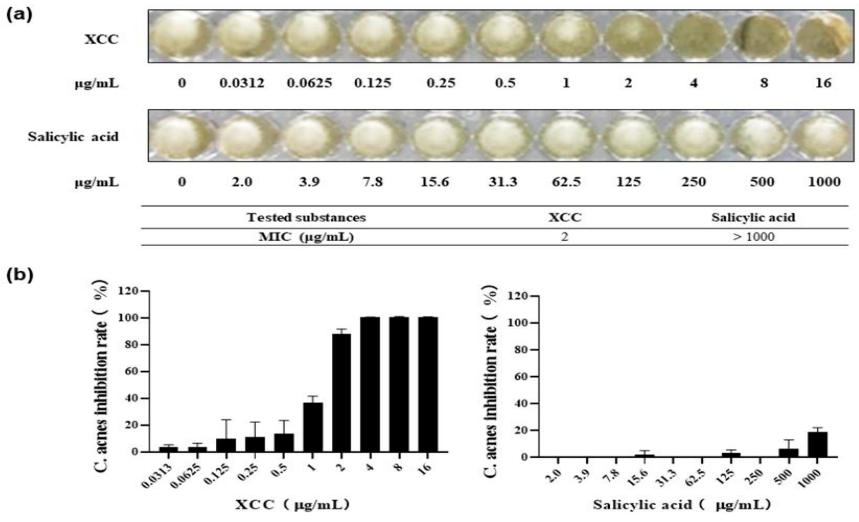

Figure 1. Effects of XCC on C. acnes growth. (a) The growth of C. acnes, treated with XCC or salicylic acid for 48 h, was detected by MIC, with the values presented below. (b) C. acnes inhibition rate of XCC or salicylic acid is depicted.

Effects of XCC on proinflammatory cytokines induced by C. acnes, TNF-α/IFN-γ or LPS

To investigate the effects of XCC on cell viability without inducing cytotoxicity, we conducted an MTT assay, which revealed no cell death at doses below 3 μg/mL after 48 h of XCC treatment (data not shown). Consequently, we selected concentrations of 0.2, 0.4, and 0.8 μg/mL of XCC for all further experiments. C. acnes-triggered cytokine activation is crucial for the progression of skin inflammation. We evaluated the impact of XCC on C. acnes-induced inflammation in HaCaT cells. Treatment with XCC led to a dose-dependent decrease in both mRNA and secretion levels of IL-6 and IL-8, as illustrated in Fig. 2a and 2c. Additionally, following 24 h of XCC treatment, there was a significant reduction in the mRNA and secretion levels of IL-6 and IL-8 in HaCaT cells stimulated with TNF-α/IFN-γ (Fig. 2b and 2d). We also investigated whether XCC could inhibit inflammation induced by C. acnes or LPS in Raw264.7 cells and PBMCs, respectively. In Raw264.7 cells exposed to C. acnes, XCC reduced the mRNA levels of IL-1β and iNOS (Fig. 3a). Furthermore, XCC decreased NO secretion in Raw264.7 cells, induced by either LPS or C. acnes (Fig. 3b). We assessed the effects of XCC on LPS-induced inflammation in PBMCs obtained from healthy individuals. XCC significantly downregulated the secretion levels of IFN-γ, IL-1β, IL-17A, and IL-23, with inhibitory effects noticeable at 0.2 μg/mL and almost complete at 0.8 μg/mL (Fig. 3c). These results underscore the potent inhibitory activities of XCC on the proinflammatory cytokine production under various stimulus conditions.

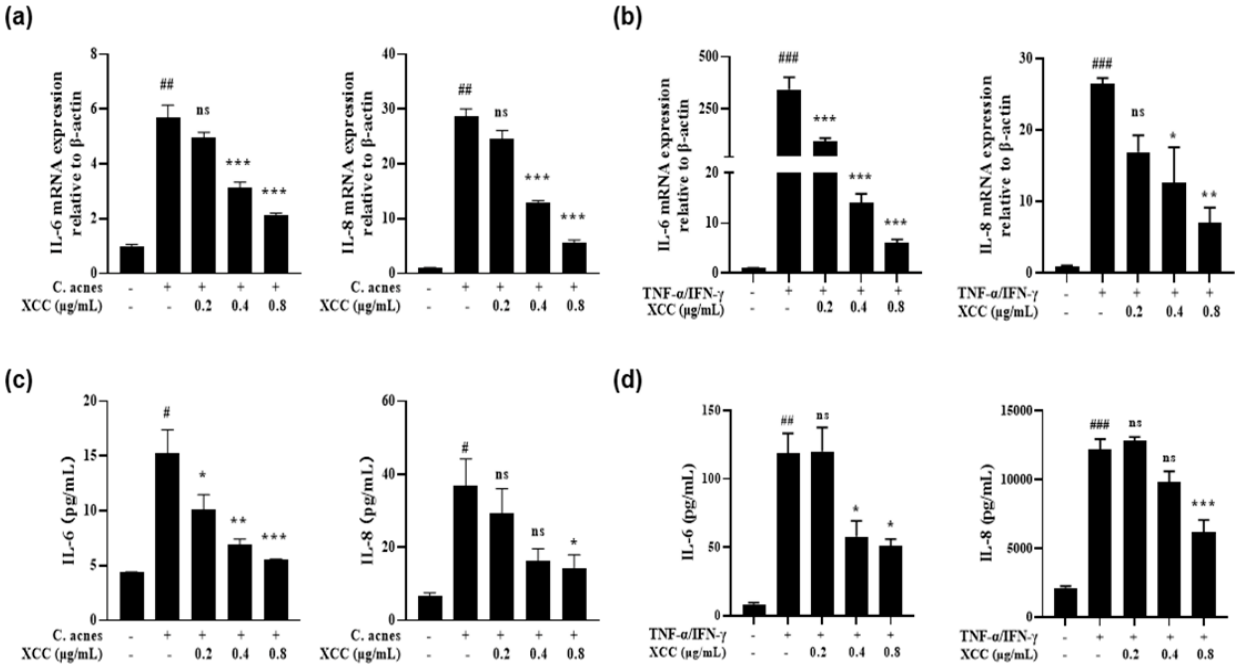

Figure 2. XCC on the production of proinflammatory cytokines in HaCaT cells. Cells underwent stimulation using C. acnes or TNF-α/IFN-γ, with or without various concentrations of XCC treatment for 24 h. Levels of IL-6 and IL-8 mRNA were quantified using qPCR (a and b). Secretion levels of IL-6 and IL-8 were evaluated via ELISA (c and d). $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ compared to the untreated group, and $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared to the stimulated group without XCC treatment.

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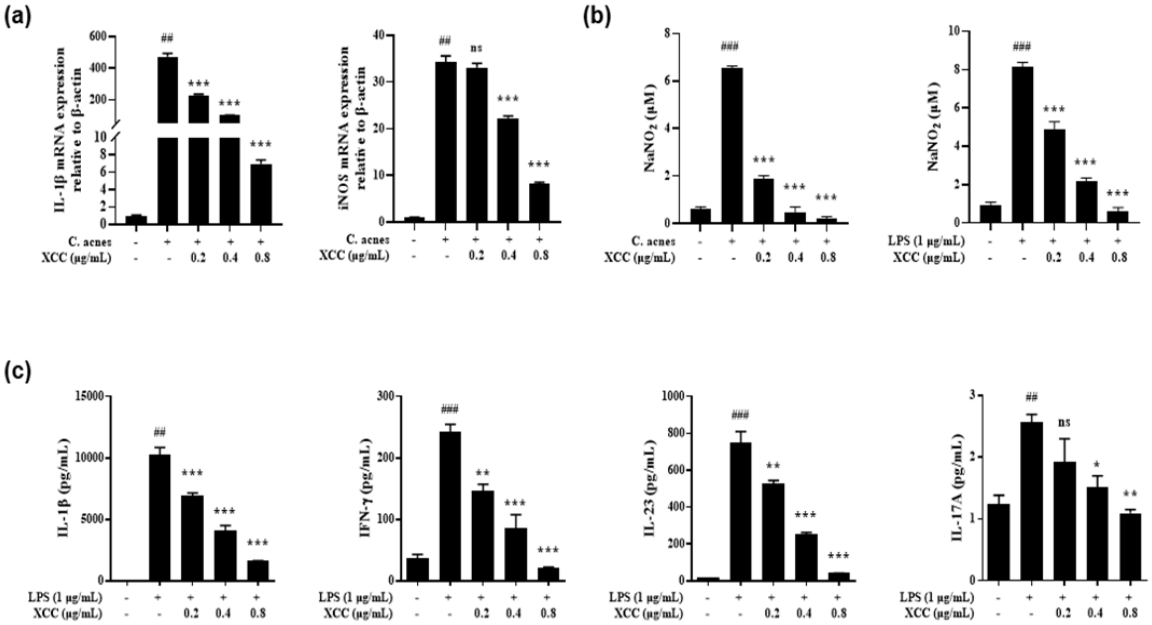

Figure 3. XCC on proinflammatory cytokine production in Raw264.7 and PBMC cells. (a) Raw264.7 cells were stimulated by C. acnes in the presence or absence of various concentrations of XCC for 24 h. Levels of IL-1β and iNOS mRNA were measured via qPCR. NO secretion was detected by Griess reagent (b). (c) PBMCs underwent stimulation with LPS with or without different concentrations of XCC for 24 h. The secretion levels of IL-1β, IFN-γ, IL-23, and IL-17A were quantified by ELISA. $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ compared to the untreated group, and $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared to the stimulated group without XCC treatment.

Effects of XCC on allergic reactions

Mast cells (MCs) function as key effectors and immunomodulators in allergic responses, particularly IgE-mediated skin allergies and the resulting pruritus. The secretion of β-hexosidase and histamine from MCs is a well-established method for evaluating mast cell degranulation in vitro. In this study, we noted that β-hexosidase and histamine expression levels were significantly elevated in DNP-IgE-sensitized RBL-2H3 and BMMCs cells upon stimulation with DNP-HSA, demonstrating an enhanced degranulation response and confirming the successful establishment of a mast cell activation model. As illustrated in Fig. 4a and 4b, the administration of XCC led to a notable reduction in both β-hexosidase activity and histamine levels.

The allergic model was established by applying MC903 topically daily (Fig. 5a). Figures 5b and 5c display representative images from the normal control (NC), model (MC903), 0.01% XCC, 0.1% XCC, and TAC groups, along with photomicrographs of ear tissue sections stained with toluidine blue (TB). The model group exhibited increased ear swelling, elevated mast cell counts, and heightened scratching behaviors. The 0.01% and 0.1% XCC groups demonstrated statistically significant differences when compared to the model group (Fig. 5d-f). ELISA results indicated that treatment with 0.1% XCC led to a reduction in serum IgE production in MC903-treated mice (Fig. 5g). Overall, the therapeutic efficacy of XCC was found to surpass that of the TAC group. These findings suggest that XCC effectively decreases mast cell numbers and scratching behaviors while inhibiting mast cell degranulation, thereby highlighting its anti-allergic properties.

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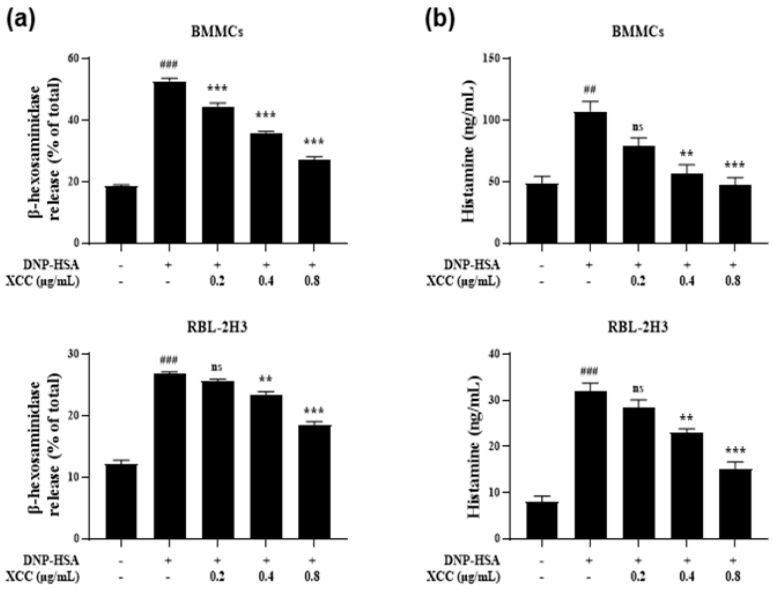

Figure 4. XCC on the release of active mediators from mast cells. IgE-sensitized RBL-2H3 and BMMC cells were incubated with XCC for 1 h prior to stimulation with DNP-HSA for 1.5 or 4 h, respectively. (a) The total release of β-hexosaminidase from both the cell supernatant and lysis buffer was quantified using a substrate chromogenic assay. (b) Histamine was determined by ELISA. $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ compared to the untreated group, and $^{**}p < 0.01$, $^{***}p < 0.001$ compared to the stimulated group without XCC treatment.

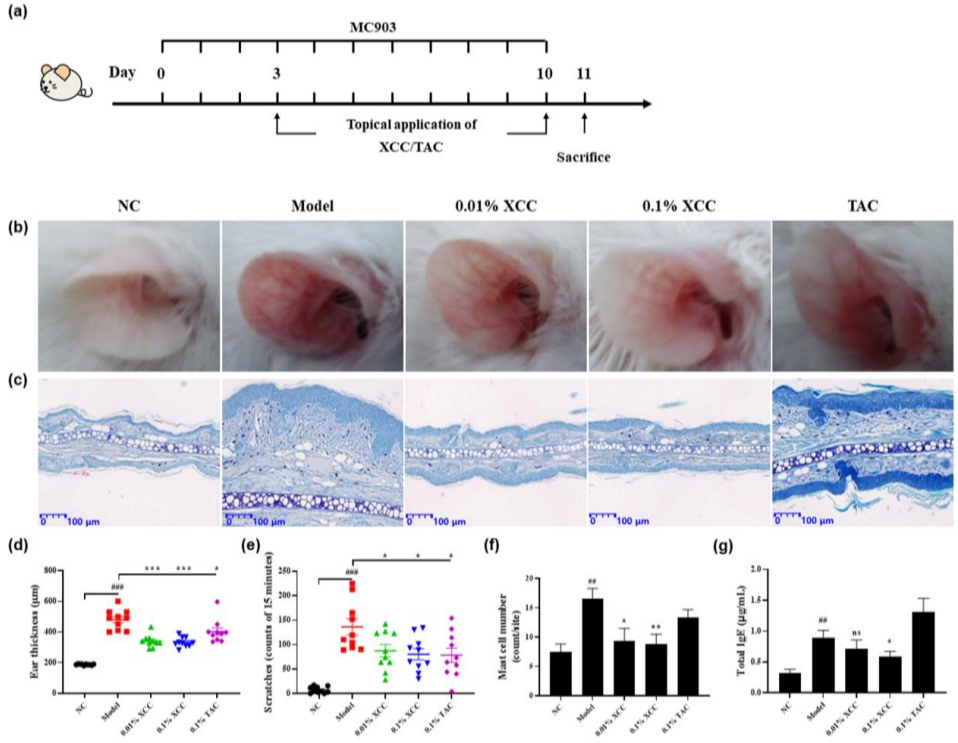

Figure 5. XCC on calcipotriol (MC903)-induced anaphylaxis. XCC or TAC was topically applied to the right ear following the establishment of the MC903 model for 8 days. (a) Experimental scheme for the MC903-induced anaphylaxis model. (b) Representative features of mouse ears at the experiment's endpoint. (c) Ear tissue sections stained with TB. (d) Ear swelling in mice measured using a digital caliper on day 11. (e) Number of scratches during a 15 min period in each mouse group. (f) MCs in TB-stained sections were calculated from 6 fields. (g) Total IgE levels in mouse serum were determined by ELISA. $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ compared to NC; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared to Model.

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Effects of XCC on antioxidation

The external environment can lead to cellular oxidation, resulting in the production of free radicals that accelerate skin aging. Consequently, strategies aimed at reducing free radical production, eliminating age-related metabolites, and enhancing antioxidant enzyme activity have emerged as effective methods for delaying skin aging. In this experiment, the oxidative properties of H_2O_2 were harnessed to induce cellular oxidative stress. Cells were preincubated with XCC for 1 h before being stimulated with 1000 μM H_2O_2 for 12 h. The results indicated that H_2O_2 increased ROS levels in the cells; however, the ROS levels were significantly harnessed following XCC treatment (Fig. 6).

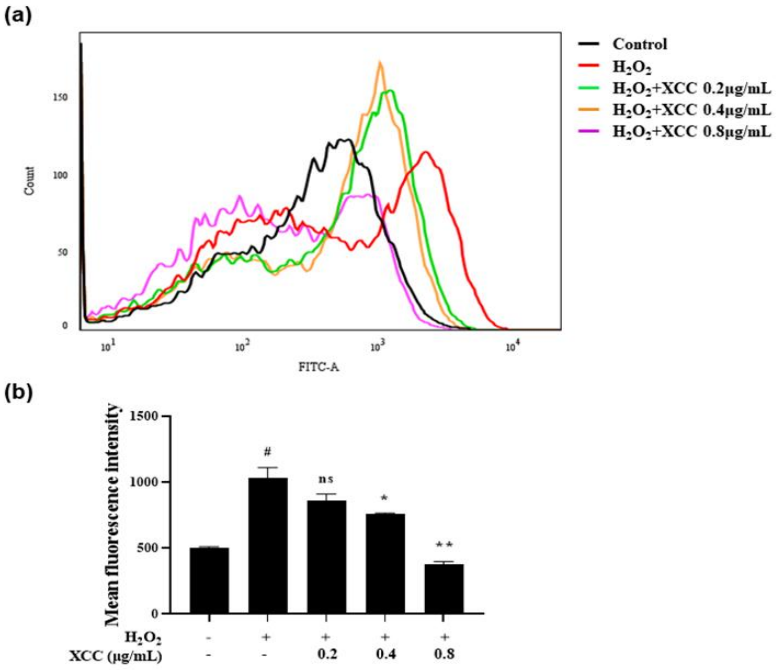

Figure 6. XCC on ROS in HaCaT cells. Cells in the experimental group were pre-incubated with varying concentrations of XCC for 1 h prior to stimulation by H_2O_2, with or without additional XCC for 12 h. The content of ROS was detected by flow cytometry (a), and (b) showing the mean fluorescence intensity. $^{\#}p < 0.05$ compared to the untreated group, and $^{*}p < 0.05$, $^{**}p < 0.01$ compared to the stimulated group without XCC treatment.

Discussion

Natural products continue to serve as the primary source of pharmacologically active candidates for skin care and treatment. Increasing interest has emerged in integrating natural plants into skincare formulations, owing to their established safety and efficacy in cosmetic applications. Natural products confer various beneficial effects on the skin, including antimicrobial, anti-inflammatory, anti-allergic, anti-aging, moisturizing, whitening, and regenerative properties (Sampath Kumar et al., 2024; Mohd Zaid et al., 2022). XCC, derived from the leaves and stalks of *Isodonis Japonicus*, has previously demonstrated multiple pharmacological activities in treating chronic skin inflammation. This observation has prompted us to further explore its potential benefits for skin health.

Human skin hosts commensal bacteria with low virulence. However, when skin integrity is compromised, this microbial balance can be disrupted, leading to the proliferation of pathogenic microbes, including Candida spp., Malassezia spp., and Staphylococcus aureus. The latter frequently causes wound infections and can lead to complex skin disorders (Zhang et al., 2024b). Additionally, the overgrowth of high-virulence C. acnes strains significantly contributes to acne vulgaris (Kolar et al., 2019). Seborrheic dermatitis is associated with Malassezia species, which hydrolyze free fatty acids and trigger immune responses (Adalsteinsson et al., 2020). Consequently, natural products exhibiting antibacterial properties are valuable in skin care and

may aid in preventing bacterial resistance to antibiotics. Our study indicated that the inhibition of C. acnes growth was enhanced at higher concentrations of XCC. Previous research has reported that *Isodon japonicus* displays inhibitory activities against various bacterial strains, including S. aureus, Candida albicans, and Pseudomonas aeruginosa (Zhou et al., 2014; Tanaka et al., 2014). Considering that mild acne is typically treated with salicylic acid, a keratolytic agent (Li et al., 2023), we found that salicylic acid had significantly weaker inhibitory effectiveness against C. acnes compared to XCC. Furthermore, isotretinoin, which is a first-line acne treatment, has an MIC of 80 μg/mL against C. acnes (Raza et al., 2013), indicating that XCC has superior antibacterial properties relative to both salicylic acid and isotretinoin.

The skin is inevitably influenced by external factors, including ultraviolet radiation, chemicals, and allergens. These irritants can provoke inflammatory or allergic responses, typically manifesting as redness, swelling, fever, pain, or itching. Such symptoms arise from alterations in immune cell activity or imbalances at the molecular and biochemical levels. IL-1β, predominantly produced by macrophages and monocytes, mediates both acute and chronic inflammation by activating innate and adaptive immune responses (Bou-Dargham et al., 2017). IL-1β prompts expression of IL-8, a key IL-1 target gene. Concurrently, IL-8 secretion is stimulated via interactions with TLR-2 and TLR-4 expressed in human keratinocytes, contributing to the development of inflammatory lesions (Nagy et al., 2005; Portugal-Cohen et al., 2024). IL-17, especially IL-17A, affects keratinocyte immune function by inducing the release of antimicrobial peptides and chemokines such as IL-8 and CXCL1, accumulating neutrophils to augment inflammation (Brembilla et al., 2018; Solá et al., 2023). Our observations indicate that XCC inhibits the release of pro-inflammatory mediators (e.g., IL-6, IL-8, IL-1β, IL-17A, and NO) across various cell types, indicating a substantial anti-inflammatory effect. Notably, XCC's comprehensive anti-inflammatory properties play a pivotal role in disrupting the interaction between keratinocytes and immune cells, thus obstructing the feedback loop in inflammatory signaling.

Allergic responses are prominent in itch dermatoses, such as atopic dermatitis and urticaria. Indeed, allergens exacerbate dermatitis flares by enhancing IgE-mediated reactivity (Mittermann et al., 2016). MCs, equipped with the high-affinity IgE receptor (FcεRI), act as crucial effector cells in these reactions (Hanieh et al., 2017). Upon activation, MCs release various mediators, notably histamine, which is the most extensively studied pruritogen. The substantial release of pruritogens triggers itching and activates alarm signals (e.g., TSLP, IL-25, IL-33), perpetuating the itch-scratch cycle (Yang and Kim, 2019). Our findings demonstrated that the secretion of allergic mediators like β-hexosidase and histamine were all suppressed, alongside reductions in scratching behavior and swelling. These effects were linked to the inhibition of mast cell proliferation and IgE release, suggesting a potential therapeutic role in managing various allergic skin conditions.

The skin barrier serves as the primary defense against external toxins, radiation, and pathogenic assaults. Oxidative stress leads to the deterioration of the skin barrier, accelerating skin aging. ROS generated by oxidative stress influence keratinocytes to produce pro-inflammatory cytokines, perpetuating a vicious cycle of increased ROS production (Zhang et al., 2024a). XCC has demonstrated the ability to eliminate ROS showing benefits in protecting the skin barrier and delaying the aging process. Furthermore, there exists a complex interaction among a compromised skin barrier, microbiome imbalances, and immune dysregulation. Pathogenic microbial invasions can damage the skin barrier and incite inflammation; conversely, these inflammatory responses can further worsen barrier integrity, cause itching, and lead to dysbiosis (Langan et al., 2020). We propose that the varied pharmacological effects of XCC could aid in repairing the skin barrier.

XCC demonstrates various biological activities, including antibacterial, anti-inflammatory, and antiallergic properties. However, the underlying mechanisms and their interrelationships remain poorly understood. Advanced research utilizing omics and bioinformatics is essential to elucidate these aspects. Additionally, the potential adverse effects of XCC across different skin types warrant investigation. Extensive animal and human studies will be necessary to establish a safe dosage range. Another challenge with natural plant extracts is the stability of their content and activity across different batches. For the future development and application of XCC, quantifying the key active substances to ensure batch consistency and stability is essential.

While XCC holds promise as an extract, several limitations persist in this study. Firstly, the human skin

environment is highly complex and differs significantly from the induced conditions utilized in this research. Hence, the observed impacts of XCC on human flora, inflammation, allergies, and skin curative effects must undergo clinical validation. Secondly, while the long-term stability and safety of XCC are recognized, they have not been exhaustively examined. Consequently, additional long-term studies are imperative. Lastly, the underlying mechanism of XCC's effect observed in this study warrants further investigation.

Conclusion

In summary, our study comprehensively analyzed the pharmacological activities of XCC from multiple perspectives to elucidate its potential applications in skincare and the treatment of skin diseases. The experimental outcomes, supported by the existing literature, confirmed that XCC exhibits significant antimicrobial properties that help prevent skin infections. Its anti-inflammatory effects are primarily evidenced through the suppression of various inflammatory mediators in keratinocytes and immune cells. Additionally, XCC has been shown to alleviate allergic skin symptoms by reducing mast cell counts, mitigating swelling, and decreasing histamine and IgE release. Furthermore, XCC has been shown to reduce ROS levels. Future research will aim to uncover the mechanisms underlying XCC's diverse biological activities. We will also explore the safe dosage range and establish quality standards for the key active compounds in XCC.

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Conflict of Interest: None.

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Contribution of Authors

Yunhui Yu & Fei Yang: Conceived idea and designed the experiments.

Dong Li, Biyan Zhang & Ling Li: Performed the experiments and collected data.

Ling Li & Biyan Zhang: Analyzed and interpreted the data.

Biyan Zhang & Ling Li: Reviewed literature, wrote, edited and revised the manuscript.

Minjie Zhang: Data curation and technical support.

Yu Cao, Yunsen Li & Zijun Chen: Conceived the idea and supervised the experiments.

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