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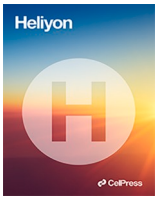

Research article

Therapeutic effects of the Qingre-Qushi recipe on atopic dermatitis through the regulation of gut microbiota and skin inflammation

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A R T I C L E I N F O

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A B S T R A C T

Accumulating evidence has highlighted a strong association between gut microbiota and the occurrence, development, prevention, and treatment of atopic dermatitis (AD). The regulation of gut microbial dysbiosis by oral traditional Chinese medicine (TCM) has garnered significant attention. In the treatment of AD, the TCM formula Qingre-Qushi Recipe (QRQS) has demonstrated clinical efficacy. However, both the therapeutic mechanisms of QRQS and its impact on gut microbiota remain unclear. Thus, our study aimed to assess the efficacy of QRQS and evaluate its influence on the composition and diversity of gut microbiota in AD animal models. First, we investigated the therapeutic effect of QRQS on AD using two animal models: filaggrin-deficient mice (Flaky tail, ft/ft) and MC903-induced AD-like mice. Subsequently, we explored its influence on the composition and diversity of gut microbiota. Our results demonstrated that QRQS treatment ameliorated the symptoms in both ft/ft mice and MC903-induced AD-like mice. It also reduced the levels of serum IgE and pro-inflammatory cytokines, including IL-1β, IL-4, IL-5, IL-9, IL-13, IL-17A, and TNF-α. Furthermore, QRQS remarkably regulated gut microbiota diversity by increasing *Lactobacillaceae* and decreasing *Bacteroidales*. The inflammatory factors in peripheral serum of ft/ft mice showed a close correlation with gut microbiota, as determined using the Spearman correlation coefficient. Additionally, PICRUSt analysis revealed an enrichment in ascorbate and aldarate metabolism, fatty acid metabolism and biosynthesis, and propanoate metabolism in the QRQS group compared to the ft/ft group. Finally, we identified liquiritin as the primary active ingredient of QRQS using ultra-high-performance liquid chromatography-high-

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resolution mass spectrometry (UPLC-HRMS). Our findings revealed that QRQS improved AD-like symptoms and alleviated skin inflammation in ft/ft and MC903-induced mice. This suggests that modulating the gut microbiota may help elucidate its anti-inflammation activation mechanism, highlighting a new therapeutic strategy that targets the intestinal flora to prevent and treat AD.

1. Introduction

Atopic dermatitis (AD) is a chronic, recurrent, inflammatory skin condition with a rising global incidence, currently affecting 20%–30% of the population [1,2]. This increase contributes significantly to the economic burden of healthcare. AD severely impacts the quality of life of patients and their families due to its chronic nature, financial demands, and the need for family involvement in treatment [3]. The etiology of AD is complex, involving epidermal barrier abnormalities, genetic factors, immunological responses, and environmental influences [4,5]. Current treatments include basic moisturizers, topical corticosteroids, calcineurin inhibitors, antihistamines, systemic corticosteroids, immune suppressants, and biologic agents [5]. However, these treatments pose challenges due to safety concerns, economic burden, and the risk of relapse post-treatment, underscoring the need for safe, effective, and cost-efficient therapeutic options [6].

Traditional Chinese Medicine (TCM) has a long history in treating skin diseases, including AD. The Qingre-Qushi Recipe (QRQS), a compound of four herbs (*Hedyotis diffusa* Willd, *Xanthium sibiricum*, *Taraxacum*, and *Sophora flavescens* Ait.), is known for its heat-clearing and dampness-dispelling properties and has been used clinically for over five decades. Our previous studies indicated an 89% response rate to QRQS in AD treatment [7]. Further research using an ovalbumin-induced AD model in BALB/c mice demonstrated QRQS's ability to alleviate AD symptoms and reduce scratching behavior by suppressing Th2 type inflammation, potentially via the IL-33/ST2 signaling pathway [8]. *In vitro*, oxymatrine, QRQS's core component, sensitized HaCaT cells to the IFN-γ pathway and downregulated MDC, ICAM-1, and SOCS1 through the activation of p38, JNK, and Akt [9]. These findings highlight QRQS's therapeutic potential in AD, though its specific anti-inflammatory mechanisms remain to be elucidated.

Oral administration is the conventional method for TCM. Recent research has identified the gut microbiota as a potential therapeutic target [10]. The gut microbiota plays a crucial role in metabolism regulation, immune modulation, and maintaining gut mucosal integrity [11]. It has been implicated in the onset and progression of AD, with abnormal gut bacterial colonization disrupting the Th1/Th2 balance and exacerbating AD[12,13]. Probiotics can correct microbiota disorders, modulate immunity, and provide antioxidant benefits, thus restoring microecological balance and aiding in AD management [13]. *Sophora flavescens*, a key component of QRQS, exhibits anti-inflammatory and antioxidant properties [14]. It has been shown to alleviate AD symptoms by reducing the Th2 inflammatory response [15,16], and significantly altering gut microbiota composition, with effects varying by dose, duration, and gender [17]. These findings suggest QRQS's potential role in AD treatment through gut microbiome regulation, warranting further investigation into its mechanisms.

This study aimed to evaluate the efficacy of QRQS in filaggrin-mutant ($Flg^{ft/ft}$) mice and MC903-induced C57BL/6 mice, focusing on its impact on inflammation and gut microbiota. We employed luminex liquid suspension chip technology to measure inflammatory cytokine levels and 16S rRNA gene sequencing to analyze gut microbiota characteristics. Furthermore, we employed PICRUSt to predict the biological function of gut microbiota. We also identified the primary active ingredient of QRQS using ultra-high-performance liquid chromatography-high-resolution mass spectrometry (UPLC-HRMS), suggesting liquiritin as the key active components in treating AD and regulating gut microbiota. This approach facilitated the study of its correlation with QRQS. Our findings suggest a novel strategy for targeting gut microbiota in AD treatment and prevention.

2. Materials and methods

2.1. Preparation of QRQS

The preparation of the QRQS extract followed the standardized process outlined in a previous study [8]. Briefly, four herbs (listed in Table 1) were immersed in water at a 1:10 w/v ratio and subjected to boiling twice, each for a duration of 1 h. Following the addition of an adequate amount of distilled water, the extract was filtered using Whatman number 2 filter paper (Maidstone, UK). The final sample was a clear, brown liquid. Tests conducted for bacteria and heat sources yielded negative results. The extract was initially concentrated at 2.7 g/mL and then diluted with ultrapure water before its application in experiments.

Table 1
The composition of QRQS.

Herb name	Latin name	English name	Use part	Amount (g)
Ku shen	*Sophora flavescens* Aiton	Lightyellow Sophora	Root	10
Bai hua she she cao	*Hedyotis diffusa Willd.*	Hedyoti scorymbosa	Whole herb with root	10
Pu gong ying	*Taraxacum mongolicum* Hand.-Mazz.	Mongolian Dandelion	Aerial parts	10
Cang er cao	*Xanthium sibiricum*	Cocklebur grass	Stem-leaf	2.5

2.2. Animals

Jackson Laboratory (Bar Harbor, ME, USA) provided ft/ft mice, while C57BL/6 mice were obtained from Lingchang Biological Technology Co., Ltd. (Shanghai, China). At the Division of Animal Resources, Shanghai University of Traditional Chinese Medicine, all mice were housed under specific pathogen-free (SPF) conditions. The facility maintained a temperature of 22 ± 2 °C and a 12-h light/dark cycle, with ad libitum access to water and food. The ft/ft mice were bred by the sibling inbreeding method, with two females and one male in one cage. At 28 weeks of age, visible symptoms such as hair loss and scratching were observed in ft/ft mice, while the appearance of AD-like lesions occurred at 32 weeks. All experiments involving live animals were approved by Shanghai University of Traditional Chinese Medicine's animal studies ethics committee (ft/ft mice, NO. PZSHUTCM18101802; C57BL/6 mice, NO. PZSHUTCM220110015).

2.3. AD-like mouse model and drug administration

A total of 40 32-week-old ft/ft mice were randomly selected and placed into six groups (n = 8) based on their dermatitis score. The groups were as follows: ft/ft group, QRQS group (3.86 g/kg, optimum dosage based on our previous study [18]), Pred group (positive control, prednisone acetate, 5 mg/kg/d, Shandong Lukang Pharmaceutical Co., Ltd., Shanghai, China), AB group (antibiotic control, Ceftriaxone Sodium, 375.5 mg/kg, Rocephin®, Roche Pharmaceutical Co., Ltd., Shanghai, China), and PB group (probiotic control, a live combined Bifidobacterium, Lactobacillus and Enterococcus capsule, 1,000 mg/kg, Bifico®, Sine Pharmaceutical Laboratories Co., Ltd., Shanghai, China), and the negative control (NC) using C57BL/6 mice. All mice were administered treatments via gavage once daily for 14 days, with the ft/ft and NC groups receiving an equivalent volume of ultrapure water.

In a separate experiment, four groups of male C57BL/6 mice (8 weeks old, 20.79 ± 0.95 g, n = 5 per group) were randomly assigned to the following treatments: NC group, MC903 group, QRQS group (3.86 g/kg) and Cetirizine (Ceti) group (positive control [19], 2 mg/kg, Zyrtec®, UCB, Pharma, Germany). The experimental groups received topical treatment with 5 nmol of MC903 (calcipotriol, Tocris Bioscience) in 50 μL of ethanol on the dorsal skin for 14 days (6 days treatment, 2 days break, 6 days treatment), while the NC group received 50 μL of ethanol as a control. The treatment groups were given gavage administration once daily for 14 consecutive days, with the NC and MC903 groups receiving a similar volume of ultrapure water.

2.4. Skin measurements and sample collection

Dermatitis score measurements were used to evaluate the severity of skin lesions on days 0, 7, and 14. Scratching behavior directed towards the back area was observed and recorded for a duration of 30 min on day 14. For a more detailed evaluation of dermatitis severity, the established Eczema Area and Severity Index (EASI) scoring system [20] was employed, involving analysis of captured images. The body weights of C57BL/6 mice were recorded on days 1, 5, 9, and 14. After evaluation on day 14, skin tissues, serum, and colonic contents were collected for analysis following euthanasia, which was carried out using CO2 at a 25% flow rate under isoflurane anesthesia.

2.5. Histopathology

For histopathological examination, lesional skin tissues were fixed in 4% formalin, embedded in paraffin, and subsequently stained with hematoxylin and eosin (H&E). Epidermal thickness was quantified by measuring four random fields from each sample using Image J software, with the average value being reported.

2.6. Enzyme linked immunosorbent assay (ELISA)

The concentration of total serum IgE in mice was determined using a commercial mouse IgE ELISA Assay Kit (PI476, Beyotime, Shanghai, China), following the manufacturer's protocol.

2.7. Luminex liquid suspension chip detection

Serum cytokine levels were analyzed by Wayen Biotechnology (Shanghai, China) using the Bio-Plex Pro Mouse Cytokine Group I Panel 23-plex (Cat. No. M60009RDPD, Bio-Rad, CA, USA), in accordance with the manufacturer's instructions.

2.8. 16S rRNA gene sequencing

Colonic content samples from ft/ft mice were sent to Majorbio Company (Shanghai, China) for DNA extraction and subsequent 16S rRNA gene sequencing. The purity of the microbial DNA extracted was verified using agarose gel electrophoresis. The V3–V4 hypervariable region of the 16S rRNA gene was amplified through Polymerase Chain Reaction (PCR). The amplified PCR products were then sequenced on Illumina MiSeq platforms at equimolar concentrations, in accordance with the manufacturer's operating manual. Additionally, 16S rRNA amplicon sequencing (16S-Seq) data was deposited at the NCBI database (BioProject PRJNA951913).

2.9. Microbiota data analysis

Initially, MiSeq-sequenced paired-end (PE) reads were merged based on overlapping regions, followed by qualitative quality control and filtering of the sequences. Subsequently, we performed OTU cluster analysis and assessed species diversity. Community structure statistics were evaluated at different taxonomic levels based on the results of the OTU clustering analysis. Partial Least Squares Discriminant Analysis (PLS-DA) was conducted to assess similarity among the groups. Linear discriminant analysis (LDA) and effect size (LEfSe) analysis were employed to identify genera distinguishing the groups and to identify distinct gut microbiota. Only genera with an LDA score greater than 2.0 and an average relative abundance exceeding 0.01% were displayed. Spearman correlation analysis was used to calculate the correlations between cytokine concentrations and microbiota populations. Additionally, we predicted the KEGG functions and pathways of the gut microbiota using PICRUSt (http://picrust.github.io/picrust/).

2.10. Ultra-high-performance liquid chromatography-high resolution mass spectrometry (UPLC-HRMS)

For the UPLC-HRMS analysis, a Vanquish UHPLC system coupled with an Orbitrap Q Exactive HFX mass spectrometer (Thermo Fisher, USA) was utilized. The mobile phase consisted of solvent A (water with 0.1% formic acid) and solvent B (acetonitrile with 0.1% formic acid), flowing at a rate of 0.3 mL/min. Following is a description of the gradient elution procedure:0–17 min, 5% B; 17.0–17.2 min, 98% B; 17.2–20 min, 5% B. The temperature in the column was maintained at 35 °C. As a general rule, mass spectrometry is conducted under the following conditions: positive ion spray voltage 3.50 kV, negative ion spray voltage −3.0 kV; sheath gas: 45 psi; aux gas: 20 psi; spare gas:0 psi; capillary temperature: 320 °C; probe heater temp: 370 °C.

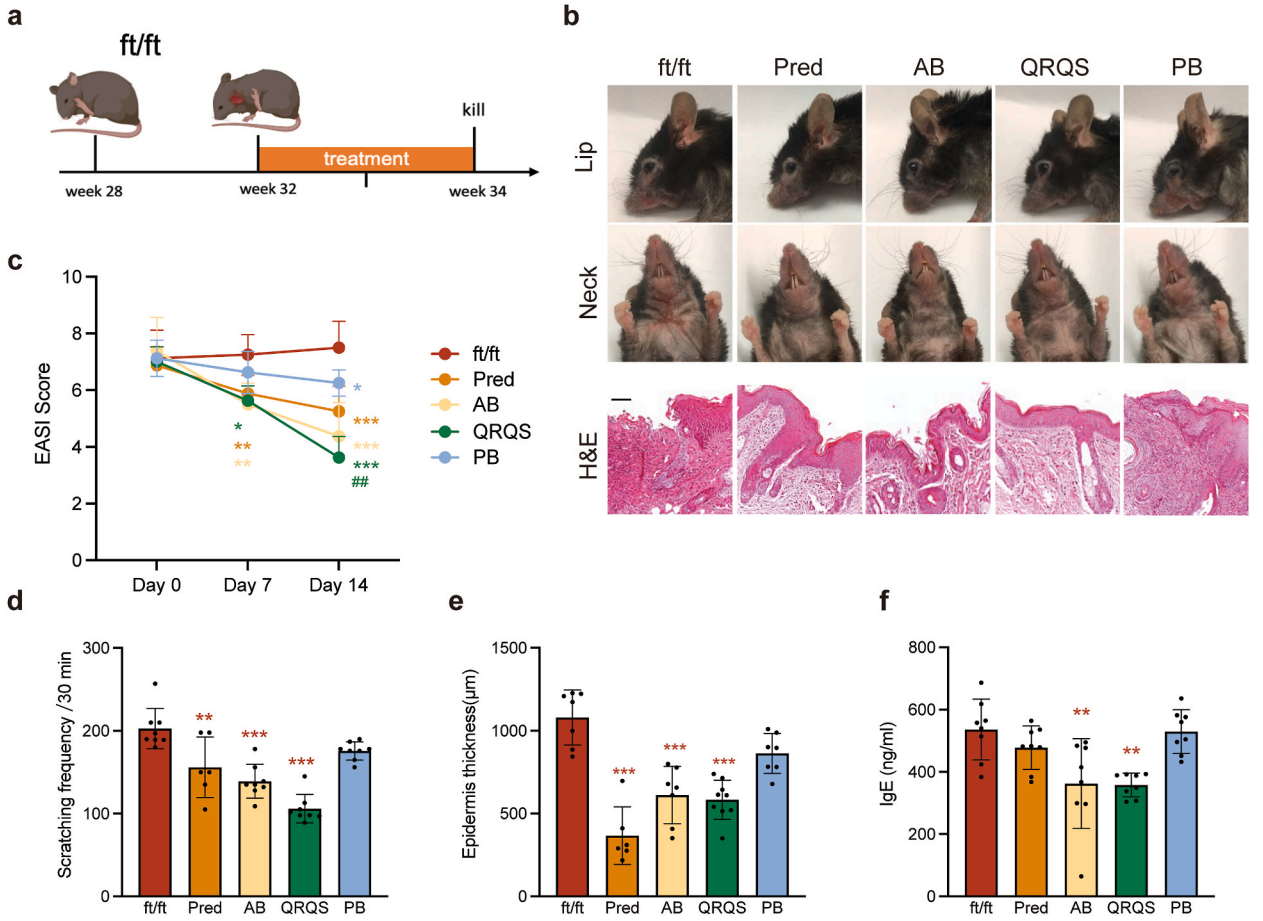

Fig. 1. QRQS attenuates AD-like symptoms in ft/ft mice. a Experimental design of ft/ft mice. Ft/ft mice developed a severe AD-like clinical phenotype at week 32. Oral administration of treatments was given daily from week 32 for 14 consecutive days. **b** Representative images of skin lesion and hematoxylin and eosin (H&E) staining in different groups, scale bar = 250 μM. **c** EASI scores, **d** scratching frequency, **e** epidermis thickness and **f** serum IgE level of each group. Means ± SD, n = 6–8. $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ vs. ft/ft group, $^{\#\#}p < 0.01$ *vs*. Pred group, one-way ANOVA with Tukey's post-test. Pred: prednisone acetate, AB: antibiotics (ceftriaxone sodium), PB: probiotics (Bifico).

2.11. High-performance liquid chromatography (HPLC)

After identifying the primary active components in QRQS via UPLC-HRMS, we conducted HPLC analysis for quantification, following the methodology outlined in our previous study [21]. For standard preparation, liquiritin (B20414, purity ≥98%), oxymatrine (B21470, purity ≥98%), and sophocarpine (B20544, purity ≥98%) were procured from Yuanye Bio-Technology (Shanghai, China). These compounds were accurately weighed and subsequently dissolved in methanol to prepare solutions at a concentration of 20 μg/mL.

2.12. Statistical analysis

GraphPad Prism 9.0 software was utilized to perform the analyses, and all data are presented as means ± standard deviation (SD). Variability between two groups was assessed using a two-tailed Student's t-test, while variation among multiple groups was evaluated using one-way analysis of variance (ANOVA) followed by Tukey's post-test. A p-value of less than 0.05 was considered statistically significant.

3. Results

3.1. QRQS ameliorated AD-like symptoms in ft/ft and MC903-induced mice

To assess the effects of QRQS on AD-like skin lesions, we employed two distinct animal models representing endogenous and exogenous conditions, respectively. The ft/ft model, an inbred strain, spontaneously develops eczematous dermatitis characterized by heightened immune responses to percutaneous antigens under SPF conditions [22]. In our previous study, we confirmed the optimum dosage of QRQS is 3.86 g/kg [18]. By week 32, these mice exhibited severe symptoms including erythema, edema, and excoriations (Fig. 1a). QRQS treatment led to marked improvements in these skin lesions (Fig. 1b). Clinical assessments showed significantly higher EASI scores in the ft/ft group compared to those treated with QRQS, Pred, AB, and PB (Fig. 1c). Additionally, QRQS was effective in alleviating itching symptoms (Fig. 1d) and reducing peripheral IgE levels (Fig. 1f). Histopathological analysis revealed epidermal/dermal proliferation and inflammatory cell infiltration in the ft/ft group, which were notably reduced following QRQS treatment (Fig. 1e).

In addition, we employed a second experimental AD mouse model induced by TSLP overexpression in mouse skin with topical MC903 administration [23–25]. QRQS also demonstrated significant efficacy in alleviating AD-like symptoms in these MC903-induced mice (Supplementary Fig. S1). These results collectively indicate that oral administration of QRQS effectively reduces AD-like symptoms in both models.

3.2. QRQS suppressed the pro-inflammatory cytokines expression in ft/ft mice

AD is characterized by immunological dysregulation and abnormal inflammation. While earlier studies have focused on the Th2 immune bias in AD, recent research has broadened to include abnormalities in Th17/Th22/Treg pathways [26]. In this context, we explored the potential of QRQS to modulate T cell-mediated immunological responses in ft/ft mice. Utilizing a liquid phase chip method, we measured cytokine levels in both QRQS-treated and untreated ft/ft mice. The serum levels of various cytokines (IL-1β, IL-4,

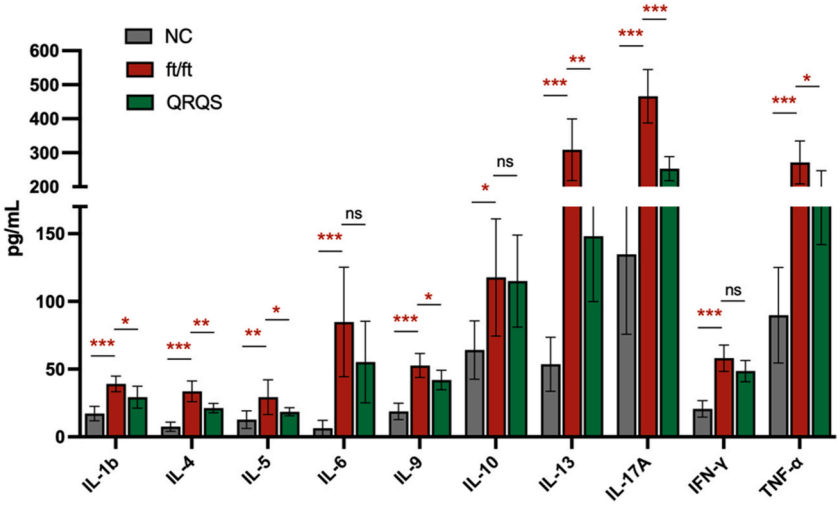

Fig. 2. QRQS inhibits the level of inflammatory factors. Comparison of inflammatory factors in serum of different groups. Means ± SD, n = 8. *$p <$ 0.05, **$p < 0.01$, and ***$p < 0.001$, one-way ANOVA with Tukey's post-test.

IL-5, IL-6, IL-9, IL-10, IL-13, IL-17A, IFN-γ, and TNF-α) were significantly higher in ft/ft mice (Fig. 2). However, QRQS treatment notably decreased the levels of IL-1β, IL-4, IL-5, IL-9, IL-13, IL-17A, and TNF-α compared to the untreated ft/ft group. These results suggest that QRQS mitigates AD symptoms by dampening both Th2 and Th17 immune responses.

3.3. QRQS regulated the diversity and composition of the gut microbiota in ft/ft mice

To assess the effect of QRQS treatment on gut microbiota diversity and abundance in ft/ft mice, we conducted 16S rRNA gene sequencing on their colonic contents. Initially, α-diversity was evaluated using the Shannon, Simpson, Chao, and Ace indexs to determine species richness and evenness (Fig. 3a). The Shannon, ACE, and Chao indexs, indicative of microbial community richness, showed a significant decrease in the AB group. Notably, the QRQS and PB groups exhibited a lower Shannon index than the AB group, yet it was significantly higher in comparison. The ACE and Chao indexs did not show significant changes in the QRQS and PB groups. The Simpson index, reflecting microbiota uniformity, indicated no significant alterations in the ft/ft mice due to QRQS and PB treatments. Further, PLS-DA analysis at the OTU level revealed distinct microbiota compositions among the different groups, with a clear separation between the ft/ft group and others (AB, PB, and QRQS) along the longitudinal axis (Fig. 3b). The AB group was distinctly separated from the PB and QRQS groups, which showed no differentiation at the OTU level (Fig. 3b).

Comparative analysis using a reference database facilitated the classification of gastrointestinal microbiota. Taxonomy-based comparisons highlighted variations in species abundance among the four groups. For instance, the relative abundance of *Lactobacillaceae* was significantly higher in the QRQS and PB groups compared to the ft/ft group, while *Bacteroidales* abundance was lower (Fig. 3c).

To identify specific taxa associated with ft/ft and QRQS treatment at the taxonomic level, we conducted LEfSe analysis to determine differential microbial branches. Dominant bacteria in the ft/ft group included *Turicibacter*, *Ruminococcaceae*, *Erysipelotrichia*, *Erysipelotrichales*, and *Erysipelotrichaceae*. Conversely, the QRQS group predominantly featured *Faecalitalea*, *Alistipes*, *Corynebacteriaceae*, and *Corynebacterium* (Fig. 3d). Additionally, 19 intestinal microbiota were enriched in the QRQS group compared to the ft/ft group,

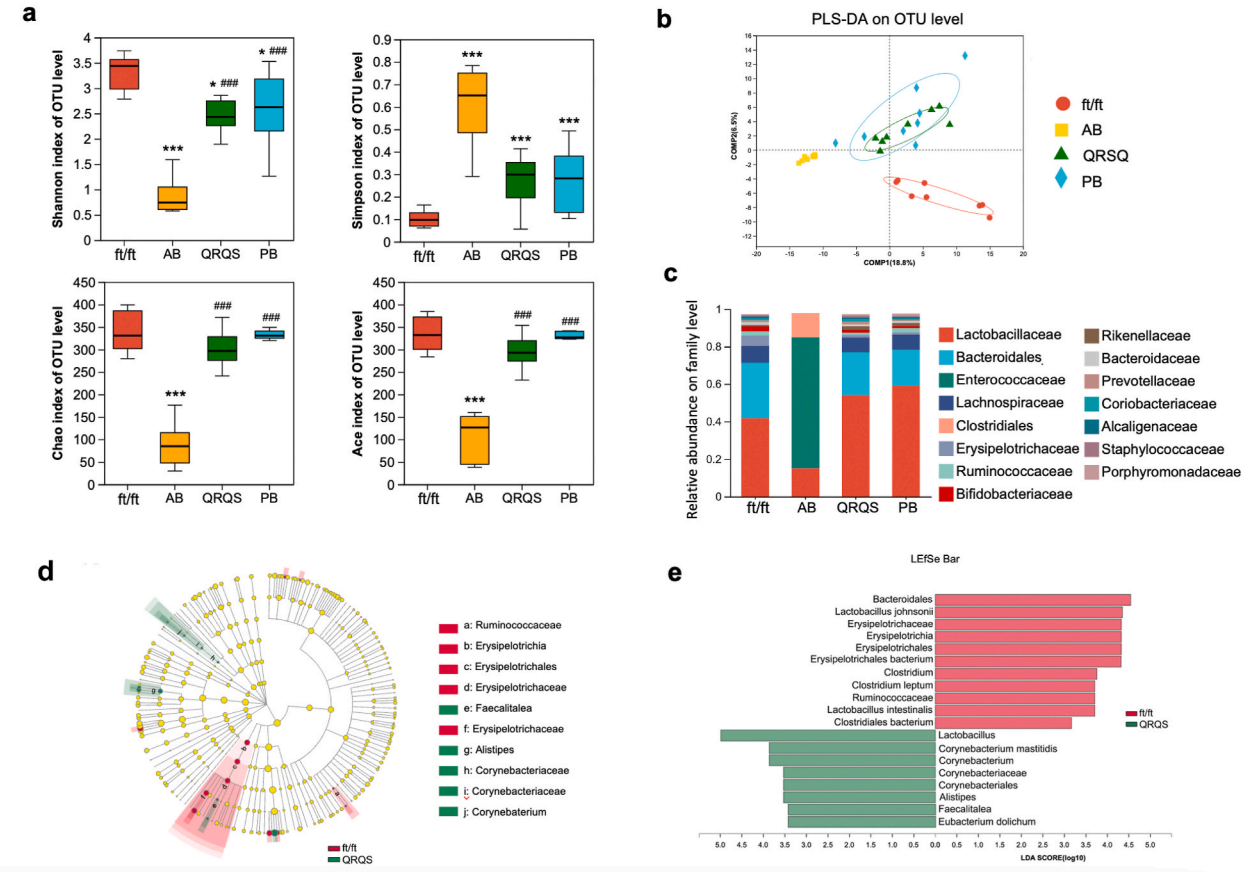

Fig. 3. Effect of QRQS on the diversity and composition of gut microbiota in ft/ft mice. **a** α-diversity analysis including Shannon, Simpson, Chao and Ace indexs on OTU level. **b** PLS-DA score plot on OTU level. **c** The heatmap of microbiota of each groups based on family level. **f** Taxonomic cladogram based on the on the linear discriminant analysis effect size (LEfSe) analysis. **e** Linear discriminant analysis (LDA) score of ft/ft group *vs.* QRQS group. n = 6–8, $^{*}p < 0.05$, and $^{***}p < 0.001$ *vs.* ft/ft group, $^{\#\#\#}p < 0.001$ *vs.* AB group, Kruskal-Wallis H test. AB: antibiotics (ceftriaxone sodium), PB: probiotics (Bifico).

including *Lactobacillus*, *Corynebacterium mastitidis*, *Corynebacterium*, *Corynebacteriaceae*, *Corynebacteriales*, *Alistipes*, *Faecalitalea*, and *Eubacterium dolichum* (Fig. 3e). These findings suggest that QRQS treatment significantly alters the gut microbiota composition in ft/ft mice, potentially contributing to its anti-AD effects through the regulation of beneficial gut microbiota such as *Lactobacillaceae*.

3.4. Correlation between gut microbiota and inflammation cytokines

Our study identified *Lactobacillaceae* as the predominant microbiota associated with QRQS treatment. Known for their use as probiotics, *Lactobacillaceae* have been linked to various health benefits, including pathogen defense and immune system activation [27, 28]. To explore the relationship between different gut microbiota and inflammatory cytokines in AD, we conducted Spearman correlation analysis. Fig. 4 displays the hierarchical clustering of Spearman's correlation coefficients, comparing the ft/ft and QRQS groups to evaluate the influence of inflammatory cytokines on the overall microbial community.

Our analysis revealed significant associations between specific microbial communities and cytokines, highlighting their potential role in AD pathogenesis. Key inflammatory cytokines in AD, such as IL-4, IL-5, and IL-13, showed notable correlations with certain microbiota. *Erysipelotrichaceae* was positively correlated with IL-4, *Lachnoclostridium* with IL-5, and *Bilophila* with IL-13. In contrast, negative correlations were observed between *Lachnospiraceae*, *Firmicutes*, and IL-4. Additionally, a notable correlation was found between IL-17A and gut microbiota; *Erysipelotrichaceae* and *Bacteroidales* both exhibited a positive association with IL-17A. These findings underscore the intricate interplay between gut microbiota and cytokines in the pathogenesis of AD.

3.5. Predicted biological function of gut microbiota

To investigate the functional characteristics of the gut microbiota associated with QRQS treatment, we utilized PICRUSt for comparative analysis of gastrointestinal microbiota functions. The gut microbiota in the QRQS group was predicted to participate in various functions, categorized into five major groups encompassing 51 functional subcategories, with a particular focus on metabolic pathways (Fig. 5a). Notably, there was significant involvement in specific metabolic pathways, such as propanoate metabolism, prodigiosin biosynthesis, and vitamin B6 metabolism (Fig. 5b). A comparative analysis of the predicted KEGG pathways between the microbiota of the QRQS and ft/ft groups was performed (Fig. 5). These findings collectively suggest that QRQS may primarily exert its anti-AD effects through the modulation of lipid and amino acid metabolism, while also demonstrating probiotic-like biological functions.

3.6. Identification of the active components in QRQS

To elucidate the active constituents and ensure the quality control of QRQS at a concentration of 2.7 g/mL, we conducted UPLC-HRMS analysis (Fig. 6a). This analysis identified multiple active substances in QRQS, detailed in Supplementary Table S3. Specifically, we quantitatively analyzed three key components in the raw herbal materials using HPLC. The concentrations were determined as

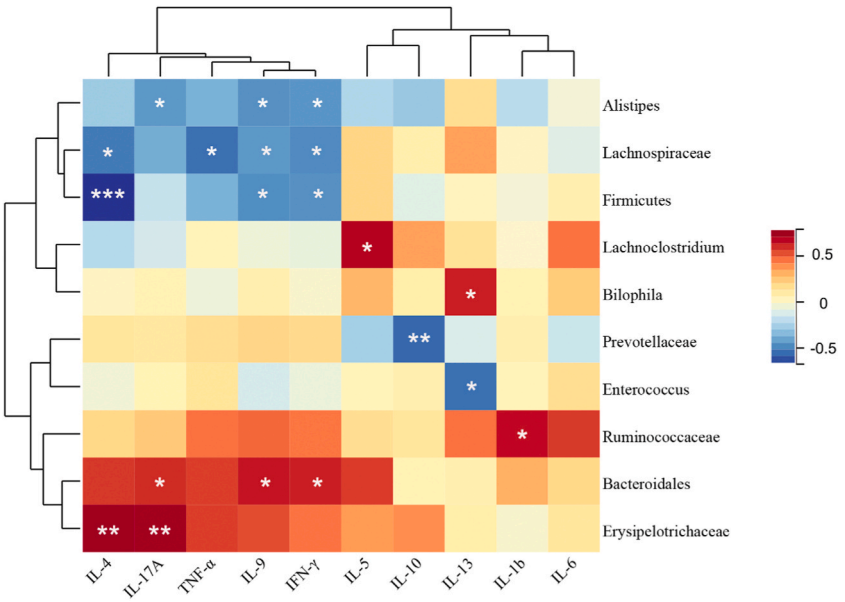

Fig. 4. Heatmap of Spearman's rank correlations of gut microbiota between serum level of inflammatory cytokines. Positively correlated samples are shown in red color and negative in blue. $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

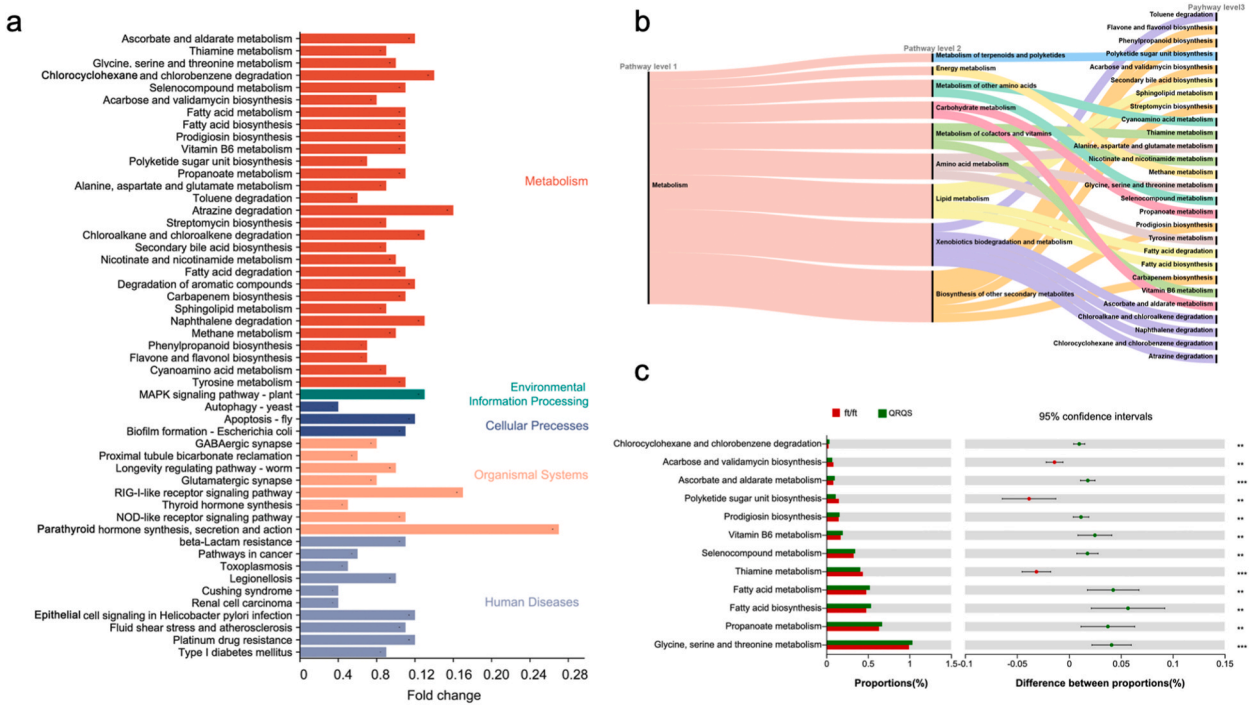

Fig. 5. Functional analysis of the gut microbiota using PICRUSt. **a** Differential pathways presented in QRQS and ft/ft groups with PICRUSt analysis. **b** Sankey diagram of 3 level classifications of differential metabolism pathway. **c** Top 12 differential pathways identified in the ft/ft group *vs.* QRQS group. Bar plots on the left side displayed the proportion of each KEGG pathway. Dot plots on the right show the differences in mean proportions between the two indicated groups using *p*-values, $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$.

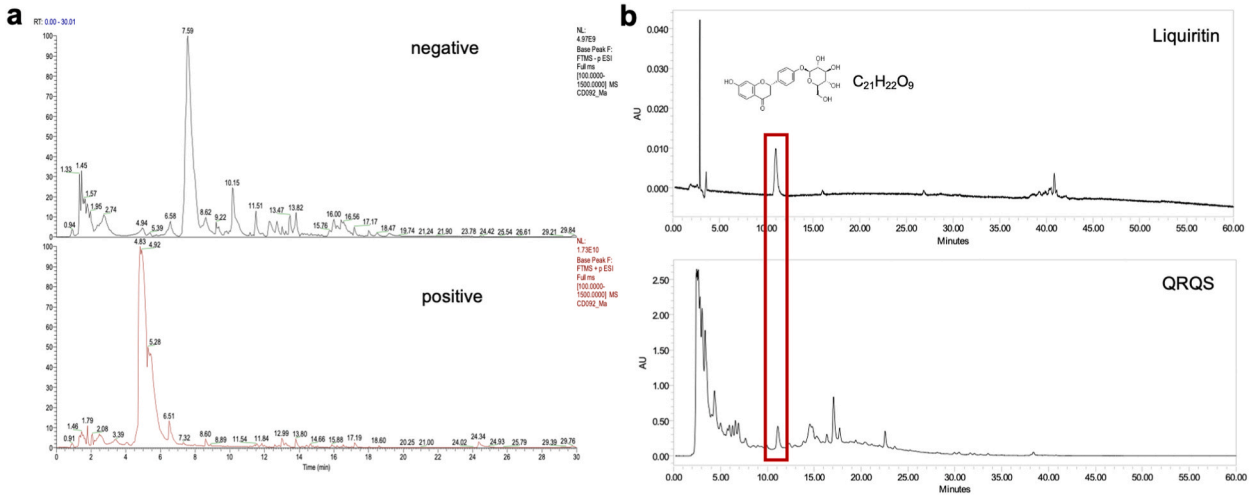

Fig. 6. Quality control analysis of the QRQS formula. **a** UPLC-HRMS chromatograms of QRQS in positive and negative modes. **b** HPLC chromatogram of QRQS extract and liquiritin as standard compounds.

follows: liquiritin at 397.74 μg/g, oxymatrine at 314.34 μg/g, and sophocarpine at 44.91 μg/g in raw medicines (Fig. 6b, Supplementary Fig. S4). The findings of this study reveal that QRQS contains a wide array of components, among which liquiritin appears to be a principal active constituent. This suggests that liquiritin could be the essential element in QRQS, contributing significantly to the improvement of AD and the regulation of gut microbiota.

4. Discussion

AD is a chronic and recurrent intractable skin disease. While biological agents have been employed in AD treatment, their long-term efficacy and potential risks remain unknown [29]. Previous research has shown that oral administration of QRQS effectively treats AD patients [7]. QRQS administration has been found to reduce inflammation cytokines and the IL-33/ST2 signaling axis associated with AD in ovalbumin-induced mice [8]. In our research, we utilized both the ft/ft and MC903-mediated AD-like mouse models. We found that QRQS ameliorated AD-like skin lesions and decreased levels of inflammatory factors, including IL-1β, IL-5, IL-6, IL-9, IL-10, IL-13, IL-17A, and TNF-α. Through 16S rRNA gene sequencing, we determined that QRQS enhances gut microbiota diversity. Additionally, UPLC-HRMS analysis identified liquiritin as a primary active component in QRQS, known for its potent anti-inflammatory properties [30]. In this study, we present evidence demonstrating the effects of QRQS on skin inflammation and gut microbiota in experimental animals. Our findings suggest a clinically safe and effective natural product-based treatment strategy for AD, providing valuable insights for future AD therapies that target gut microbiota.

AD is characterized by chronic and recurrent skin conditions, where inflammatory responses play a pivotal role in its pathogenesis. Our findings add to this understanding by illustrating a potential link between inflammatory markers and gut microbiota. Elevated serum levels of IL-4, IL-17, IL-9, and IFN-γ in our study correlated positively with the abundance of *Erysipelotrichaceae* and *Bacteroidales*, implicating these microbiota in exacerbating AD symptoms. The exact mechanisms through which gut microbiota influence inflammatory factors remain elusive. However, it is recognized that gut microbiota can have systemic effects, either by modulating host metabolites or by producing their own metabolites. These microbiotas generate a diverse array of metabolites and signaling molecules, such as short-chain fatty acids, non-ribosomal peptides, oligosaccharides, amino acid metabolites, glycolipids, and post-translationally modified peptides [31,32]. This interaction, wherein gut microbiota metabolites impact skin and immune cells, is known as the gut-skin axis [33]. An imbalance in gut microbiota can lead to increased intestinal permeability and compromise the integrity of the intestinal barrier. This disruption allows harmful metabolites to enter the bloodstream and reach the skin, where they provoke a strong Th2 response. Such a response exacerbates the symptoms of skin lesions in AD patients [34], highlighting the critical role of gut microbiota in disease progression.

While biological agents are common in AD treatment, their long-term safety and efficacy are still under investigation [4]. Conversely, numerous studies have validated the efficacy of orally administered Chinese herbal medicine in AD management [35]. Significantly, this approach has also been shown to modulate and substantially alter the structures of gut microbiota communities [35–37]. This presents a safer and more comprehensive treatment strategy, emphasizing the crucial role of gut microbiota diversity in AD management—a factor commonly neglected in standard AD therapies. In addition to changes in skin microbiota, AD patients often exhibit reduced gut microbiota diversity [38,39]. Probiotics are known to enhance the gut environment, balance immune responses, and regulate metabolic functions [40–42]. In our study, QRQS remarkably ameliorated AD-like skin lesions and reduced levels of various inflammatory factors in mouse models. In addition, we observed that QRQS regulated both the diversity and composition of the gut microbiota in ft/ft mice. This effect was marked by an increased relative abundance of *Lactobacillaceae* and a decreased relative abundance of *Bacteroidales*. *Lactobacillaceae*, belonging to the *Lactobacillus* family, is a commonly known probiotic bacteria. It not only promotes the innate immune system but also reduces serum IgE levels, maintains a balance between Th1 and Th2 responses, and strengthens the skin, immune, and intestinal barriers [43–45]. Conversely, *Bacteroidales*, found in higher abundance in the gut microbiome of AD patients, are strongly associated with allergic diseases when present at high levels in the colon [46]. Interestingly, the overall composition of gut microbiota showed no significant difference between the QRQS and probiotic groups. However, while antibiotics had similar effects on skin symptoms, they adversely affected the gut microbiota structure in ft/ft mice. It is noteworthy that adding *Lactobacillus* alone did not alter the gut microbiota in AD patients [47,48]. Therefore, achieving a dynamic balance between probiotics and pathogenic bacteria by altering the gut microbiota structure is crucial in the treatment of AD.

We employed PICRUSt to investigate the biological function of gut microbiota in the QRQS group and discovered a predominant clustering in metabolic pathways. QRQS appears to exert anti-AD effects by modulating lipid and amino acid metabolism, including propanoate biosynthesis. Short-chain fatty acids (SCFAs) are known to directly influence cells involved in allergic reactions. Research indicates that SCFAs can inhibit the FcεRI-mediated signaling cascade, thereby attenuating allergic responses [49–51]. Notably, fecal samples from AD patients show reduced levels of SCFAs, particularly butyrate and propionate [52,53]. There is evidence suggesting the therapeutic potential of butyrate or propionate in treating allergic airway diseases and food allergies [54,55]. Studies have also demonstrated that exposure to butyrate or propionate may decrease IL-4 release, potentially alleviating allergic reactions and restoring skin barrier integrity [56]. This is corroborated by findings that patients with IgE-mediated food allergies have lower fecal levels of acetate, propionate, and butyrate [57]. Additionally, QRQS influences the metabolism of glycine, serine, threonine, ascorbate, aldarate, and thiamine, indicating that changes in these pathways might be linked to AD pathophysiology, although relevant literature on this subject is currently scarce.

Monomers are key active components in traditional Chinese medicine. In our study, we identified three primary active monomers in QRQS: liquiritin, oxymatrine, and sophoridine. Liquiritin has been demonstrated to mitigate inflammatory infiltration and angiogenesis in rheumatoid arthritis, as well as modulate inflammatory pathways [30]. It also has general inhibitory effects on *Clostridium* and *Bacteroides* [58]. The anti-inflammatory properties of oxymatrine have been extensively studied in various inflammatory models [59,60]. Notably, oxymatrine alleviates skin inflammation symptoms in AD mice in a dose-dependent manner by decreasing serum levels of TNF-α, IgE, IL-4, IL-6, and IL-17 [16]. It also effectively mitigates inflammatory bowel disease by fostering the proliferation of beneficial bacterial strains and simultaneously suppressing pathogenic bacteria [61]. Sophoridine also exhibits anti-inflammatory effects, having been found to suppress the production of TNF-α, PGE2, and IL-8 in both *in vivo* and *in vitro* experiments [62]. These results collectively indicate that QRQS has the potential to effectively address AD via a multitude of pathways.

Our study has uncovered that QRQS can mitigate AD through its anti-inflammatory properties. More importantly, we have discovered its probiotic-like effect on the gut microbiota, potentially attributed to the metabolic products of its multi-herbal composition. Our findings emphasize that, beyond ameliorating specific symptoms, TCM can also balance the body's systems, aligning with the holistic philosophy of TCM. The multifaceted, multi-target strategy inherent in TCM formulas like QRQS can culminate in more holistic and comprehensive treatment outcomes.

5. Conclusion

Our research underscores the promise of QRQS as an integrated treatment strategy for AD, demonstrating its capacity to alleviate skin lesions in diverse models, including ft/ft mice and MC903-induced AD-like mouse models. QRQS stands out not only for its ability to dampen inflammatory cytokines but also for its proficiency in modulating gut microbiota, a function that mirrors the benefits of probiotics. The anti-inflammatory properties of QRQS are potentially tied to its modulation of intestinal metabolites, suggesting an innovative approach to AD therapy via gut microbiota regulation. This finding prompts an intriguing consideration: could QRQS, as a synergistic compound, offer more substantial benefits in AD management compared to its individual constituents like liquiritin? Future investigations are warranted to explore this possibility, with a particular emphasis on the role of QRQS in the regulation of gut microbiota and their metabolites, which are critical in both the treatment and prevention of AD.

Data availability statement

The sequence data was deposited in NCBI database (BioProject PRJNA951913). Data included in article/supp. material/referenced in article.

CRediT authorship contribution statement

Fang Shen: Writing – original draft. **Chunjie Gao:** Writing – original draft, Investigation. **Mingxia Wang:** Investigation. **Xiaojie Ding:** Data curation. **Hang Zhao:** Data curation. **Mi Zhou:** Formal analysis. **Jingyi Mao:** Formal analysis. **Le Kuai:** Writing – review & editing, Visualization. **Bin Li:** Writing – review & editing, Resources. **Dongming Wang:** Writing – review & editing, Investigation, Formal analysis. **Huimin Zhang:** Resources, Funding acquisition, Conceptualization. **Xin Ma:** Writing – review & editing, Writing – original draft, Resources, Investigation, Conceptualization.

Declaration of competing interest

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

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Appendix T. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e26063.

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