

# *Lonicera japonica* Thunb. and its characteristic component chlorogenic acid alleviated experimental colitis by promoting *Lactobacillus* and fecal short-chain fatty acids production

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## Funding information

National Natural Science Foundation of China, Grant/Award Number: 32101932; Key Research and Development Program of Zhejiang Province, Grant/Award Number: 2021C02018; Fundamental Research Funds for the Central Universities, Grant/Award Numbers: 226-2022-00173, 226-2022-00215; Jilin Science and Technology Plan Project, Grant/Award Number: 20211-055350

## Abstract

Ulcerative colitis is intricately linked to intestinal oxidative stress and dysbiosis of the gut microbiota. *Lonicera japonica* Thunb. (LJ) is a traditional edible and medical flower in China, and chlorogenic acid (CGA) is one of its characteristic components. However, it remains unclear whether gut microbiota plays a role in the therapeutic effects of LJ and CGA on colitis. Here, we first observed that oral administration of LJ and CGA for 3 weeks dramatically promoted the growth of *Lactobacillus* and fecal short-chain fatty acids (SCFAs) production in healthy mice. Subsequently, the alleviating effects of LJ and CGA on colitis were explored with a dextran sulfate sodium-induced colitis mice model. The intervention of LJ and CGA notably alleviated inflammation, intestinal barrier impairment, and oxidative stress in colitis and led to a significant elevation in *Lactobacillus* and fecal SCFAs. Eventually, the key role of gut microbiota and their metabolites on the therapeutic effects was validated by performing fecal microbiota transplantation and sterile fecal suspensions transplantation from LJ and CGA-treated healthy mice to colitis mice. Our findings demonstrated that consumption of LJ and CGA could benefit the host both in healthy condition and colitis. The beneficial effects were attributed to the improvement of the endogenous antioxidant system and promotion of the probiotic *Lactobacillus* and SCFAs production. Our study highlighted the great potential of LJ and CGA to be consumed as functional foods and provided novel mechanisms by which they alleviated colitis.

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## KEY WORDS

chlorogenic acid, colitis, gut microbiota, *Lactobacillus*, *Lonicera japonica* Thunb, short-chain fatty acids

## 1 | INTRODUCTION

Inflammatory bowel disease (IBD) manifests as chronic and recurrent inflammation in the gastrointestinal tract, encompassing Crohn's disease, ulcerative colitis (UC), and indeterminate colitis (Sairenji et al., 2017). The prevalence of IBD has surged in recent years, imposing enormous health and economic burdens on communities worldwide (Alatab et al., 2020). The etiopathogenesis of IBD is intricate and multifaceted, involving genetic susceptibility, environmental risk factors, intestinal barrier defects, immune dysfunction, intestinal oxidative stress, and microbial dysbiosis (Guo et al., 2020; Ramos & Papadakis, 2019; Yang et al., 2022), aspects that have not been fully elucidated. Thus, there is an urgent imperative for the development of safe and well-tolerated therapeutic strategies to achieve sustained long-term remission (Perse & Cerar, 2012).

Oxidative stress emerges when a marked imbalance exists between the production and clearance of reactive oxygen species (ROS; Pizzino et al., 2017). The inseparable relationship between inflammation and oxidative stress is widely acknowledged. In the context of IBD, there is typically an elevation in ROS levels coupled with a reduction in the concentration of related antioxidants. With the progression of UC, persistent inflammation leads to the collapse of enzymatic and non-enzymatic antioxidant defense systems equipped in colon tissues, which in turn aggravates the severity of UC. Consequently, it is firmly posited that oxidative stress plays a substantial role in the induction and progress of UC (Jena et al., 2012; Rezaie et al., 2007).

Gut microbiota refers to the trillions of microorganisms inhabiting the gastrointestinal tract (Martel et al., 2017), which play a pivotal role in the maintenance of intestinal homeostasis (D. Li et al., 2021). The onset of UC is proposed to be closely related to gut microbiota dysbiosis (Guo et al., 2020). The intestinal epithelium is shielded and protected by a mucus layer that keeps bacteria separated from the mucosa (Johansson et al., 2008). Gut microbiota dysbiosis contributes to a severe deterioration of the mucus layer, heightening susceptibility to IBD (Makki et al., 2018). A previous study demonstrated that germ-free mice colonized with the fecal microbiota from irritable bowel syndrome (IBS) patients exhibited similar intestinal and behavioral manifestations of IBS (De Palma et al., 2017). Furthermore, in recent years, fecal microbiota transplantation (FMT) has also emerged as a novel therapy to treat UC (Guo et al., 2020).

Gut microbiota-derived metabolites exert important and diverse effects on host physiology, acting as the principal molecular mediators between the microbiota and host. Profound alterations in the fecal, urinary, and serum metabolomes have been documented in IBD (Lavelle & Sokol, 2020). Among these metabolites, short-chain fatty acids (SCFAs), mainly acetate, propionate, and butyrate, are one of

the most prominent metabolites. SCFAs possess key roles in regulating host metabolism, immune system, cell proliferation, and the integrity of the intestinal barrier (Koh et al., 2016). Importantly, IBD patients showed reduced levels of SCFAs production and dominant SCFAs-producing bacteria, implicating the potential therapeutic value of SCFAs for IBD (Parada Venegas et al., 2019).

Honeysuckle (*Lonicera japonica* Thunb. [LJ]) is a traditional edible and medicinal flower in China. Modern pharmacological studies have validated that LJ possesses a broad spectrum of bioactivities such as anti-inflammatory, antioxidant, antiviral, antibacterial, and anti-tumor properties. These beneficial effects are believed to arise from the abundant bioactive phytochemicals present in LJ (Shang et al., 2011). The chemical profiles of LJ have been clearly elucidated in previous studies (Seo et al., 2012; Y. Wang et al., 2023; Ye et al., 2014). In line with *The Pharmacopoeia of the People's Republic of China 2020 Edition* (hereinafter referred to as the *Chinese Pharmacopoeia*), chlorogenic acid (CGA), 3,5-dicaffeoylquinic acid, 4,5-dicaffeoylquinic acid and luteolin-7-O-glucoside are indicator components for evaluating the quality of LJ. Previous studies have elucidated that LJ extract showed potent alleviating effects on chemically induced murine colitis (D. Liu et al., 2020; Park et al., 2013). Nevertheless, whether LJ polyphenol could modulate gut microbiota and whether gut microbiota plays a role in LJ-mediated alleviation of colitis remain rarely explored. We hypothesized that LJ and its principal component CGA may positively regulate the gut microbiota and SCFAs production both in healthy and diseased status, consequently contributing to the alleviation of inflammation and oxidative stress manifested in murine colitis.

In the present study, the modulation effects of LJ and CGA on the healthy gut microbiota were first explored. The inflammatory and antioxidant effects of LJ and CGA were next validated with a dextran sulfate sodium (DSS)-induced murine colitis model. Finally, the key role of gut microbiota in alleviating colitis was further verified through an FMT and sterile fecal suspensions (SFS)-transplantation experiment. Our findings provided novel insights into the underlying mechanisms by which LJ and CGA relieved colitis.

## 2 | MATERIALS AND METHODS

### 2.1 | Preparation, identification, and quantification of LJ extract

Fresh LJ buds were harvested in Linyi City, Shandong Province, China, in 2021. Selected buds without mechanical damage, diseases, or insects were dried by natural seasoning in a cool and dry place. Completely dried buds were ground into powder and sifted through a 60-mesh

sieve. The resulting powder was subsequently ultrasonically extracted with absolute ethanol ( $m:v = 1:5$ ) in four cycles. The suspension was combined and filtered. The filtrate was vacuum dried with a rotary evaporator (Laborota 4000-efficient, Heidolph) and then redissolved with purified water. The suspension was lyophilized, yielding LJ extract, which was stored at  $-20^{\circ}\text{C}$  for further experiment.

The dark green powder was analyzed by high-performance liquid chromatography (HPLC) conducted on a Waters e2695 HPLC System with a 2998 PDA Detector (Waters Corp.). Samples were analyzed by a Sunfire C18 ODS column ( $4.6 \times 250 \text{ mm}, 5 \mu\text{m}$ , Waters Corp.). The analytes were gradient eluted by mobile phase A (acetonitrile) and B (purified water) with the optimized program: 0–5 min, 20% A; 5–15 min, 20%–27% A, 15–16 min, 27%–40% A, 16–17 min, 40%–60% A, 17–18 min, 60%–80% A, 18–20 min, 80%–100% A, 20–21 min, 100%–20% A, 21–22 min, 20% A. The flow rate was  $1 \text{ mL/min}$ , the injection volume was  $10 \mu\text{L}$ , and the monitoring UV wavelength was set at 330 and 350 nm. Detected peaks were compared and identified with authentic standards: CGA, 3,5-dicaffeoylquinic acid, 4,5-dicaffeoylquinic acid (purity  $\geq 98\%$ , CFW Laboratories Inc.) and luteolin-7-O-glucoside (purity  $\geq 98\%$ , Shanghai Yuanye Bio-Technology Co. Ltd.)

## 2.2 | Evaluation of the anti-inflammatory capacity of LJ extract in vitro by a cell model

Evaluation of anti-inflammatory capacity was conducted according to Cao et al with modifications (Cao et al., 2018). Mouse leukemia virus-induced macrophage cell line RAW264.7 was obtained from Dalian Meilun Biotechnology Co. Ltd. RAW264.7 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, 20 mM N-(2-Hydroxyethyl)piperazine-N'-(2-ethanesulfonic acid) (HEPES), 100 U/mL penicillin and 100  $\mu\text{g/mL}$  streptomycin in a humidified incubator ( $37^{\circ}\text{C}, 5\% \text{CO}_2$ ). Cells were passaged by trypsinization when reaching the confluence of 80%–90%. Cells in the exponential growth phase were harvested and seeded in a 96-well plate at a density of  $5 \times 10^4$  cells per well. After incubation for 24 h, cells were treated with LJ extract (12.5, 25, 50, 100, 200  $\mu\text{g/mL}$ ). Cell viability was measured by a Cell Counting Kit (CCK)-8 (Dojindo China Co. Ltd.) according to the manufacturer's instructions. Doses that did not significantly affect the cell viability were selected for subsequent experiments.

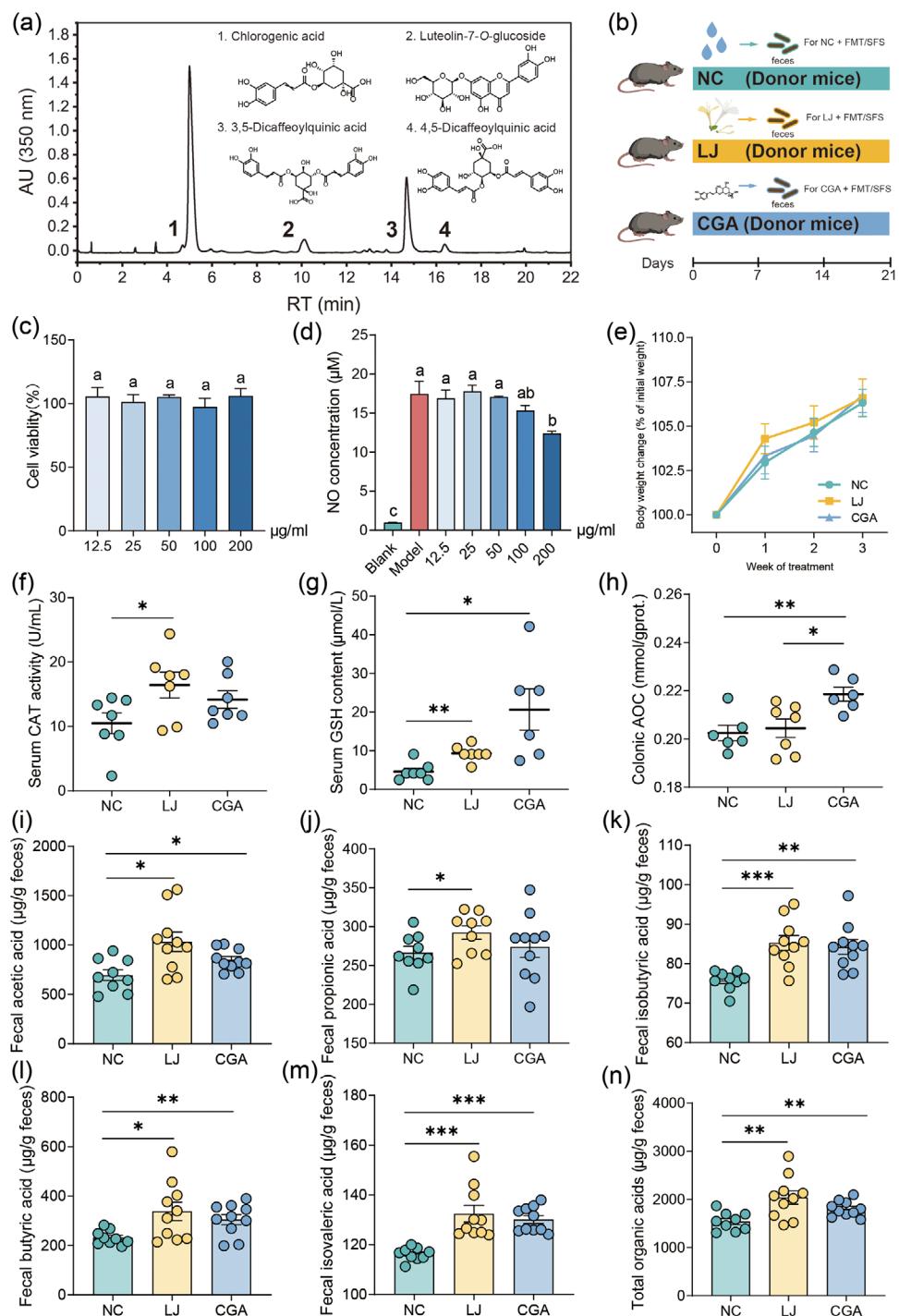
RAW264.7 cells were seeded in a 96-well plate at a density of  $3 \times 10^4$  cells per well. After incubation for 12 h, cells were treated with fresh complete DMEM medium containing different doses of LJ extract for 2 h. Then, lipopolysaccharide (LPS) at a final dose of 100 ng/mL was added to the medium, followed by another 24-h incubation. The Nitric oxide (NO) contents in the medium were detected with a NO Assay kit (Beyotime Biotechnology). Cells pretreated with Dimethyl sulfoxide (DMSO), followed with or without LPS incubation, were set as positive control and blank control. Each experiment was repeated three times independently.

## 2.3 | Animal Experiment 1: Effects of LJ extract and CGA on healthy mice

All animal experiments were conducted in accordance with the Committee on the Ethics of Animal Experiments of Zhejiang University with Permission Number ZJU20220372. Specific pathogen-free (SPF) C57BL/6 mice (6- to 8-week-old, female) were obtained from Hangzhou Medical College. All the mice were housed in a controlled environment ( $23 \pm 3^{\circ}\text{C}$ , 12 h daylight cycle) and fed with a standard chow diet (Jiangsu Xietong Pharmaceutical Bioengineering Co. Ltd.). The formula of the lab mice diet for C57BL/6J mice is listed in Table S1. After acclimation for a week, the mice were randomly divided into three groups ( $n = 10$  per group): (1) normal control group (NC group, supplemented daily with purified water); (2) LJ extract group (LJ group, supplemented daily with LJ extract with a dose of 200 mg/kg/day, 16.2 mg/kg/day equivalent to human); (3) CGA group (supplemented daily with CGA with a dose of 100 mg/kg/day, 8.1 mg/kg/day equivalent to human). Mice were treated by gavage for 21 days. The experiment scheme is displayed in Figure 1b. Body weight and food intake of the mice were recorded weekly. Of note, on Day 20, fresh mice feces of each group were collected, pooled, and weighed for subsequent FMT or SFS, which will be illustrated in detail in Animal Experiment 3. On Day 21, fresh feces of each individual mouse were collected and snap frozen in liquid nitrogen and kept at  $-80^{\circ}\text{C}$  for further use. Serum samples were separated from the blood by centrifugation. After sacrifice, mice heart, liver, spleen, kidney, and colon were dissected and weighed, snap frozen or fixed with 4% paraformaldehyde fix solution (Beyotime Biotechnology) for further use.

## 2.4 | Animal Experiment 2: Effects of LJ extract and CGA on DSS-induced colitis mice

SPF C57BL/6 mice (6- to 8-week-old, female) were obtained from Hangzhou Medical College. After 1 week of acclimation, mice were randomly divided into four groups ( $n = 8$  per group). Colitis was induced by administrating 3.5% (w/v) DSS (36–50 kDa, Coolaber) dissolved in drinking water for 7 days. The groups were as follows: (1) CON group: free access to purified water for 7 days, together with daily oral administration of water; (2) DSS group: free access to 3.5% DSS water for 7 days, together with daily oral administration of water; (3) DSS + LJ group: free access to 3.5% DSS water for 7 days, together with daily oral administration of LJ extract (200 mg/kg/day); (4) DSS + CGA group: free access to 3.5% DSS water for 7 days, together with daily oral administration of CGA (100 mg/kg/day). The experiment scheme is displayed in Figure 4a. Body weight, food, and water intake were monitored daily. Disease activity index (DAI) was evaluated by combining the parameters of weight loss, stool consistency, and rectal bleeding to assess the severity of the colitis according to previous studies (Peng et al., 2019). Rectal bleeding was evaluated with a fecal occult blood test kit (Shanghai Yuanye Bio-Technology Co. Ltd.). On Day 7, serum samples were separated from the blood by



**FIGURE 1** *Lonicera japonica* Thunb. (LJ) extract and chlorogenic acid (CGA) improved the endogenous antioxidant status and fecal short-chain fatty acids (SCFAs) production in healthy mice. (a) Representative HPLC chromatogram of LJ extract ( $\lambda = 350$  nm). (b) Study design for Animal Experiment 1. (c) Effects of LJ extract on the cell viability of RAW264.7. (d) Effects of LJ extract on the NO production in lipopolysaccharide (LPS)-simulated RAW264.7 cells. Cell assays were repeated three times independently. (e) Daily body weight changes during Animal Experiment 1 ( $n = 10$  per group). Levels of serum catalase (CAT) activity (f), serum glutathione (GSH) content (g), and colonic AOC in healthy mice (h) ( $n = 6-7$  per group). Levels of fecal SCFAs production in healthy mice, including acetic acid (i), propionic acid (j), isobutyric acid (k), butyric acid (l), isovaleric acid (m), and total organic acids (n) ( $n = 9-10$  per group). Error bars were expressed as mean  $\pm$  SEM. One-way analysis of variance (ANOVA) with Tukey's multiple comparisons was used for multi-group comparisons. Different letter on the bar indicates a significance ( $p < .05$ ). Independent samples' Student's t-test (for parametric datasets) or the Mann–Whitney U test (for non-parametric datasets) was used for two groups' comparison.

\* $p < .05$ , \*\* $p < .01$ , \*\*\* $p < .001$ . AOC, antioxidant capacity; HPLC, high-performance liquid chromatography; SEM, standard error of the mean.

centrifugation. After euthanasia, the length of the mice colon was measured. Part of the colon was cut off and fixed with 4% paraformaldehyde fix solution for histological evaluation. The colonic contents and tissue of the remaining colon were separated, both of which were collected and snap-frozen in liquid nitrogen and stored in  $-80^{\circ}\text{C}$  for further use.

## 2.5 | Animal Experiment 3: FMT and SFS transplantation

FMT was conducted according to previous studies with modifications (Arifuzzaman et al., 2022; Daoust et al., 2023; Wu et al., 2021; Zeng et al., 2020). Donor mice were from Animal Experiment 1. The fecal suspensions of the donor mice were prepared as follows: Fresh fecal pellets were resuspended and homogenized in cold sterile Phosphate-buffered saline (PBS) containing 10% glycerol in an anaerobic chamber, with a final concentration of 100 mg feces/mL. Fecal slurries were centrifuged at 500 g for 3 min to remove particulate matters. For SFS, the microbiota in the fecal suspensions were heat-killed according to previous study (W.-L. Sun et al., 2021). The FMT and SFS were then aliquoted and stored at  $-80^{\circ}\text{C}$ . Each aliquot was thawed once, when performing intragastric gavage. Recipient C57BL/6 mice (6- to 8-week-old, female) were obtained from Hangzhou Medical College. After 1 week of acclimation, mice were randomly divided into six groups ( $n = 8$  per group). All mice were provided with free access to 3.5% (w/v) DSS drink water, together with daily administration of 100  $\mu\text{L}$  corresponding FMT or SFS for 6 days: (1) NC + FMT group; (2) LJ + FMT group; (3) CGA + FMT group; (4) NC + SFS group; (5) LJ + SFS group; (6) CGA + SFS group. Evaluation of colitis severity and sampling was conducted in accordance with Animal Experiment 2.

## 2.6 | Quantification of inflammatory and antioxidant indexes in the colon and serum

Colon tissues were sufficiently homogenized with cold PBS by a homogenizer (FastPrep-24 5G, MP Biomedicals). Following centrifugation, the protein content of the supernatants was quantified with a bicinchoninic acid protein assay kit (Beyotime Biotechnology). The serum LPS and Interleukin-1 beta ( $\text{IL}-1\beta$ ) concentrations were determined by enzyme-linked immunosorbent assay (ELISA) kits (Shanghai Enzyme-linked Biotechnology Co. Ltd.; Proteintech Group Inc.) as per the manufacturer's instructions. Levels of total antioxidant capacity (AOC), malondialdehyde (MDA), glutathione (GSH), and activities of total superoxide dismutase (SOD) and catalase (CAT) of the serum and colon were measured by commercial kits (Nanjing Jiancheng Bioengineering Institute) following the manufacturer's instructions.

## 2.7 | Quantification of fecal SCFAs by gas chromatography

Fecal pellets (approximately 100 mg) were homogenized with cold absolute ethanol ( $m: v = 1: 5$ ) by a homogenizer (FastPrep-24 5G). The fecal slurries were ultrasonically extracted in cold water for 30 min, stood in  $-20^{\circ}\text{C}$  for 30 min, and subsequently centrifugated at 13,000 g ( $4^{\circ}\text{C}$ ) for 15 min. The supernatants were pipetted for gas chromatography (GC) analysis. The GC analysis was operated with a DB-624 capillary column ( $30 \text{ m} \times 0.32 \text{ mm} \times 1.8 \mu\text{m}$ , Agilent J&W Scientific). Helium (purity  $\geq 99.999\%$ ) was used as carrier gas with a constant flow rate of 1.0 mL/min, and the split ratio was 5:1. For the analysis, 1  $\mu\text{L}$  samples was detected by the optimized temperature gradient: the initial temperature was set at  $100^{\circ}\text{C}$ , then raised to  $200^{\circ}\text{C}$  at a rate of  $20^{\circ}\text{C}/\text{min}$ , and held at  $200^{\circ}\text{C}$  for 5 min. The SCFAs were identified and quantified with authentic standards: acetic acid, propionic acid, butyric acid, isobutyric acid, valeric acid, and isovaleric acid (purity  $\geq 99.5\%$ , Shanghai Aladdin Biochemical Technology Co. Ltd.).

## 2.8 | Histological evaluation

Mice heart, liver, spleen, kidney, and colon were fixed in 4% paraformaldehyde and embedded in paraffin after dehydration. Paraffin-embedded tissues were sectioned (4  $\mu\text{m}$  in thickness) and then stained with hematoxylin and eosin (H&E).

## 2.9 | RNA extraction and quantitative reverse transcription polymerase chain reaction (PCR) (RT-qPCR)

Total RNA of colonic tissues was extracted with a commercial kit (Easy-do Biotechnology Co. Ltd.) according to the manufacturer's instructions. The quality and concentration of the RNA were assessed by a NanoDrop (Thermo Fisher Scientific Inc.). Reverse transcription of the RNA was conducted with a commercial kit (Nanjing Vazyme Biotech Co. Ltd.). Quantitative PCR was operated on the CFX96 Touch Real-Time PCR Detection System (Bio-Rad Laboratories Inc.) with a SYBR qPCR kit (Vazyme). The primer sequences of the target genes are listed in Table S2, with  $\beta$ -actin serving as the endogenous control. The relative expressions of target genes were calculated using the  $2^{-\Delta\Delta\text{CT}}$  method.

## 2.10 | Fecal microbiota 16S rRNA gene sequencing

Total microbial genomic DNA was extracted from mice feces or colonic contents with an E.Z.N.A. stool DNA Kit (Omega BioTek). The quality of DNA was assessed through 1.0% agarose gel electrophoresis. The

hypervariable region V3-V4 of the bacterial 16S rRNA gene was amplified with primer pairs 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'). After PCR amplification, the purified amplicons were subjected to paired-end sequencing on an Illumina MiSeq PE300 platform (Illumina) according to the standard protocols by Majorbio Bio-Pharm Technology Co. Ltd.

## 2.11 | Statistical analysis

The statistical analysis was performed on IBM SPSS Statistics software (version 26.0, IBM). All data were expressed as mean  $\pm$  standard error of the mean. A Shapiro-Wilk test was performed to determine the distribution of the data. A one-way analysis of variance with Tukey's multiple comparisons (for parametric datasets) or the Kruskal-Wallis (for non-parametric datasets) test was used for multi-group comparisons. Two-group comparisons were conducted using independent samples' Student's t-test (for parametric datasets) or the Mann-Whitney U test (for non-parametric datasets). A significance level of  $p < .05$  was considered statistically significant.

For gut microbiota sequencing data, raw reads were quality-filtered and merged after demultiplexing. High-quality sequences were denoised using DADA2 plugin in the QIIME2 to generate amplicon sequence variants (ASVs). ASVs were taxonomically assigned using the naive Bayes consensus taxonomy classifier implemented in QIIME2 based on the SILVA 16S rRNA database (v138). Alpha diversity indices were calculated with Mothur v1.30.1. Principal coordinate analysis (PCoA) was performed based on Bray-Curtis dissimilarity. Co-occurrence networks were built and analyzed using Cytoscape v3.9.1 based on Spearman's rank correlation coefficient ( $|r| > .6, p < .05$ ). Significantly differentially abundant taxa between groups were identified by the linear discriminant analysis (LDA) effect size (LEfSe) with an LDA threshold of 3.0. Graphs were plotted by GraphPad Prism (version 8.0, GraphPad Software) and OriginPro 2023 Learning Edition (OriginLab Corp.).

## 3 | RESULTS

### 3.1 | Identification and quantification of characteristic components of LJ extract

According to *Chinese Pharmacopoeia*, CGA, 3,5-dicaffeoylquinic acid, 4,5-dicaffeoylquinic acid, and luteolin-7-O-glucoside are indicator components for evaluating the quality of LJ. The HPLC chromatogram of the LJ extract is shown in Figure 1a. The four compounds were identified by comparing the retention time of the peaks with authentic standards and quantified with standard curve methods. The contents of CGA, 3,5-dicaffeoylquinic acid, 4,5-dicaffeoylquinic acid, and luteolin-7-O-glucoside were  $33.38 \pm 2.33$ ,  $9.87 \pm 0.15$ ,  $1.16 \pm 0.01$  and  $1.96 \pm 0.05$  mg/g dry weight (DW), respectively. The mass fraction of the three phenolic acids and luteolin-7-O-glucoside were  $4.44\% \pm 0.03\%$  and  $0.20\% \pm 0.00\%$ , both of which have met the quality standards of LJ in *Chinese Pharmacopoeia*.

### 3.2 | LJ extract showed significant anti-inflammatory capacity in LPS-stimulated RAW264.7 cell model

The RAW264.7 cells represent a widely utilized in vitro model for screening anti-inflammatory compounds. They produce robust inflammatory response to stimuli such as LPS, culminating in the production of inflammatory mediators such as NO (Facchin et al., 2022). We first examined the possible toxicity of LJ extract on RAW264.7 cells by a CCK-8 assay. The results showed that LJ extract showed no discernible toxicity on RAW264.7 cells even under the highest experimental dose of 200  $\mu$ g/mL (Figure 1c). We further evaluated the anti-inflammatory capacity of LJ extract with an LPS-stimulated RAW264.7 model. As displayed in Figure 1d, LPS drastically induced the production of NO in RAW264.7 cells. Compared with the model group, LJ extract inhibited the production of NO in a dose-dependent manner, while only a dose of 200  $\mu$ g/mL could significantly reduce the NO production, with an inhibition rate of 29%. Taken together, these results demonstrated that LJ extract presented great anti-inflammatory effects in vitro.

### 3.3 | LJ extract and CGA improved the antioxidant status of healthy mice

CGA stands out as the characteristic compound with the highest content in LJ. To assess the impact of LJ extract and CGA on the physical condition of healthy mice, particularly their endogenous antioxidant status, we conducted a 3-week animal experiment (Figure 1b). As shown in Figure 1e, intake of LJ extract and CGA exhibited no significant influence on the body weight of the mice. To ascertain the potential toxicity of LJ extract and CGA on systematic organs (colon, heart, liver, spleen, and kidney), we weighed and further performed histological evaluation of systematic organs. As indicated in Table S3 and Figure S1, no obvious differences in any of the vital organs were found in weight and histology among the three groups. Other signs of toxicity and behavior abnormality were not observed either.

We next assessed the antioxidant status of mice serum and colon tissue. As exhibited in Figures 1f-h and S2a-e, intake of LJ extract or CGA significantly elevated the serum CAT activity, serum GSH content, and colonic AOC, compared with the NC group, suggesting that our treatment may improve the endogenous antioxidant status of health mice to some extent. Nevertheless, no significant differences were found in serum AOC, serum SOD activity, colonic GSH content, colonic SOD, and CAT activity.

### 3.4 | LJ extract and CGA modulated the fecal SCFAs production and gut microbiota composition

The content of fecal SCFAs were detected by gas chromatography. Six kinds of SCFAs were detected in healthy mice feces, and the most abundant SCFAs were acetic, propionic, and butyric acids. As depicted in Figure 1i-n, intervention of LJ extract and CGA significantly promoted the production of acetic acid, propionic acid, butyric acid, isobutyric

acid, and isovaleric acid, thus significantly increasing the total SCFAs production. However, no significance was observed in the valeric acid content among the three groups (Figure S2f).

Fecal SCFAs production is closely related with gut microbiota composition. We further explored the fecal microbial community by 16S rRNA gene sequencing, detecting a total of 535 ASVs. Bray–Curtis distance-based PCoA was first performed to visualize the gut microbiota composition differences among the three groups based on ASV level. As shown in Figure 2a, samples of LJ and CGA groups composed a cluster that was distinctly separated from the NC group. This indicated that LJ extract and CGA intervention generated a significant alteration in the gut microbiota composition (Analysis of similarities (ANOSIM):  $r = .4731$ ,  $p = .001$ ). The results of PCoA largely agreed with that of hierarchical clustering analysis (Figure 2b), where samples from the NC group clustered together and branched differently from those of the LJ and CGA groups. Interestingly, despite the apparent differences in gut microbiota composition among the three groups, no substantial differences were observed in the  $\alpha$ -diversity (reflected by Sobs and Shannon indices, Figure 2c–d), suggesting that LJ extract and CGA treatment did not result in significant changes in bacterial community diversity. Subsequently, co-occurrence network analysis was conducted based on core species (with a relative abundance ranked top 50). Co-occurrence networks capture the significant patterns in relative abundance between different taxa, either as positive or negative correlations (Dugas et al., 2018). As exhibited in Figure 2e–g, the three groups showed some different topological network features. While all the networks had similar sizes in terms of nodes, the network of the LJ group had more edges (214) and a greater network density (0.207), compared to those of the NC group (133 and 0.118) and CGA group (130 and 0.130). LJ group also had a greater number of core bacteria species (inner cycle, degree  $> 10$ ). These results suggested that LJ extract intervention led to a gut microbial community with higher complexity and stability.

We analyzed the bacterial taxonomic profiles at the phylum and genus levels to assess the effect of LJ extract and CGA on the overall gut microbiota composition. As shown in Figure 3a,b, Firmicutes and Bacteroidota were the predominant phyla of the gut microbiota at the phylum level. At the genus level, it was notable that the average relative abundance of *Lactobacillus* in the LJ and CGA groups was much higher than that of the NC group. Differentially abundant fecal bacteria were further identified by LEfSe analysis at the genus level between the NC group and the treatment group with an LDA threshold of 3.0. A total of 12 (NC vs. LJ) and 11 (NC vs. LJ) key discriminative genera were identified (Figure 3c,d). Detailed comparison of bacteria whose relative abundance ranked top 10 is exhibited in Figure 3e. Particularly, *Lactobacillus* and *Coriobacteriaceae\_UCG-002* were significantly abundant, while the relative abundance of *Streptococcus* and *Akkermansia* was significantly lower in response to LJ extract and CGA administration. Additionally, oral LJ extract significantly suppressed the growth of *Dubosiella*, *Turicibacter*, *Bacillus*, *unclassified\_c\_Bacilli*, and oral CGA-enriched *Alistipes* and depleted *Blautia*.

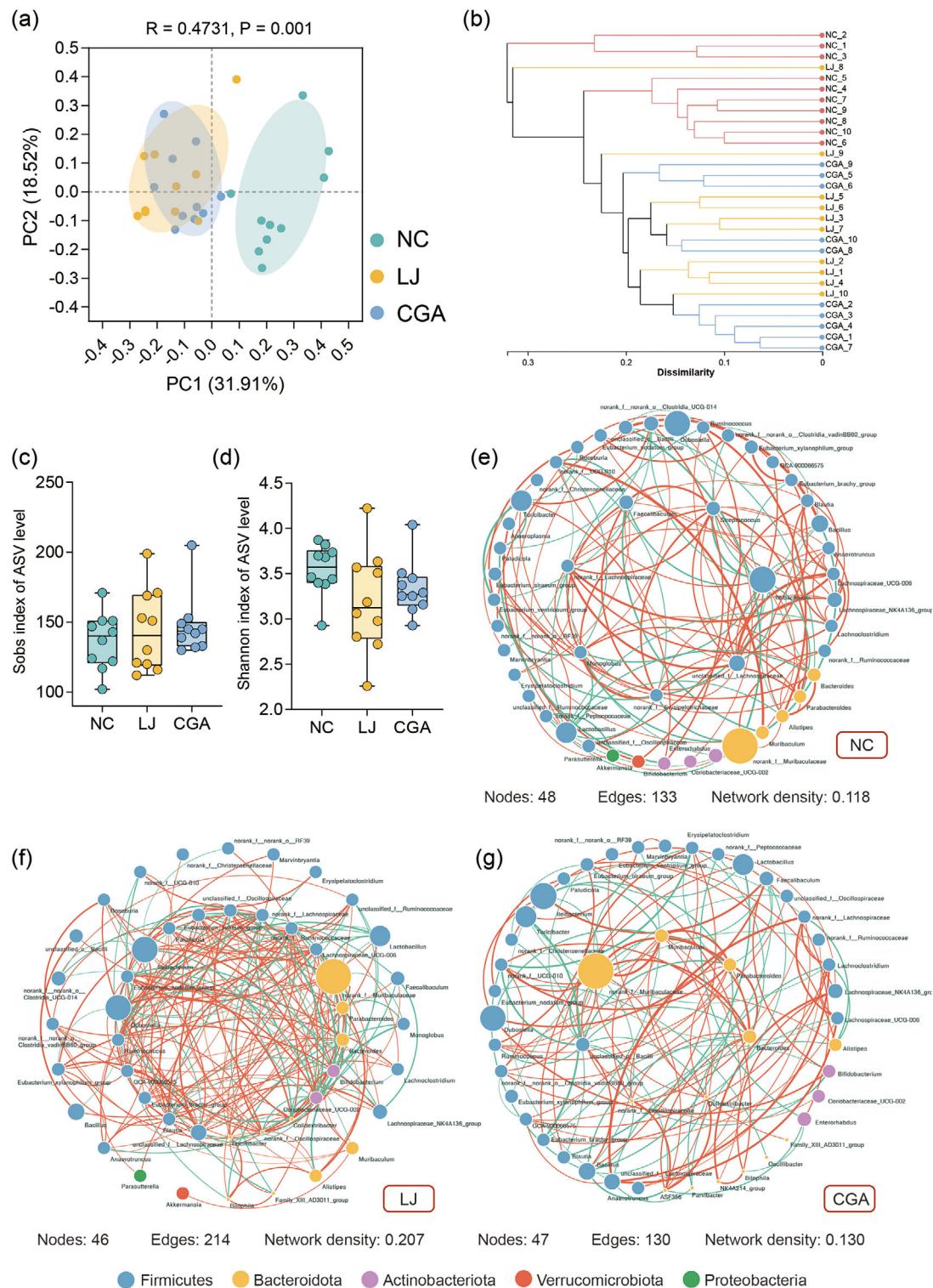
Given the close relationship between gut microbiota and SCFAs, Spearman correlation analysis was further conducted to

reveal the association between the discriminative genera and fecal SCFAs profiles. The results indicated that *Lactobacillus* and *Coriobacteriaceae\_UCG-002* were positively correlated with the content of most SCFAs (acetic acid, butyric acid, isobutyric acid, valeric acid, and isovaleric acid,  $p < .05$ ). *Alistipes* was positively correlated with isobutyric acid and isovaleric acid content ( $p < .05$ ). Nevertheless, negative correlations were found between *Blautia*, *Dubosiella*, *Akkermansia*, *Streptococcus*, and SCFAs production, despite that some correlations were insignificant.

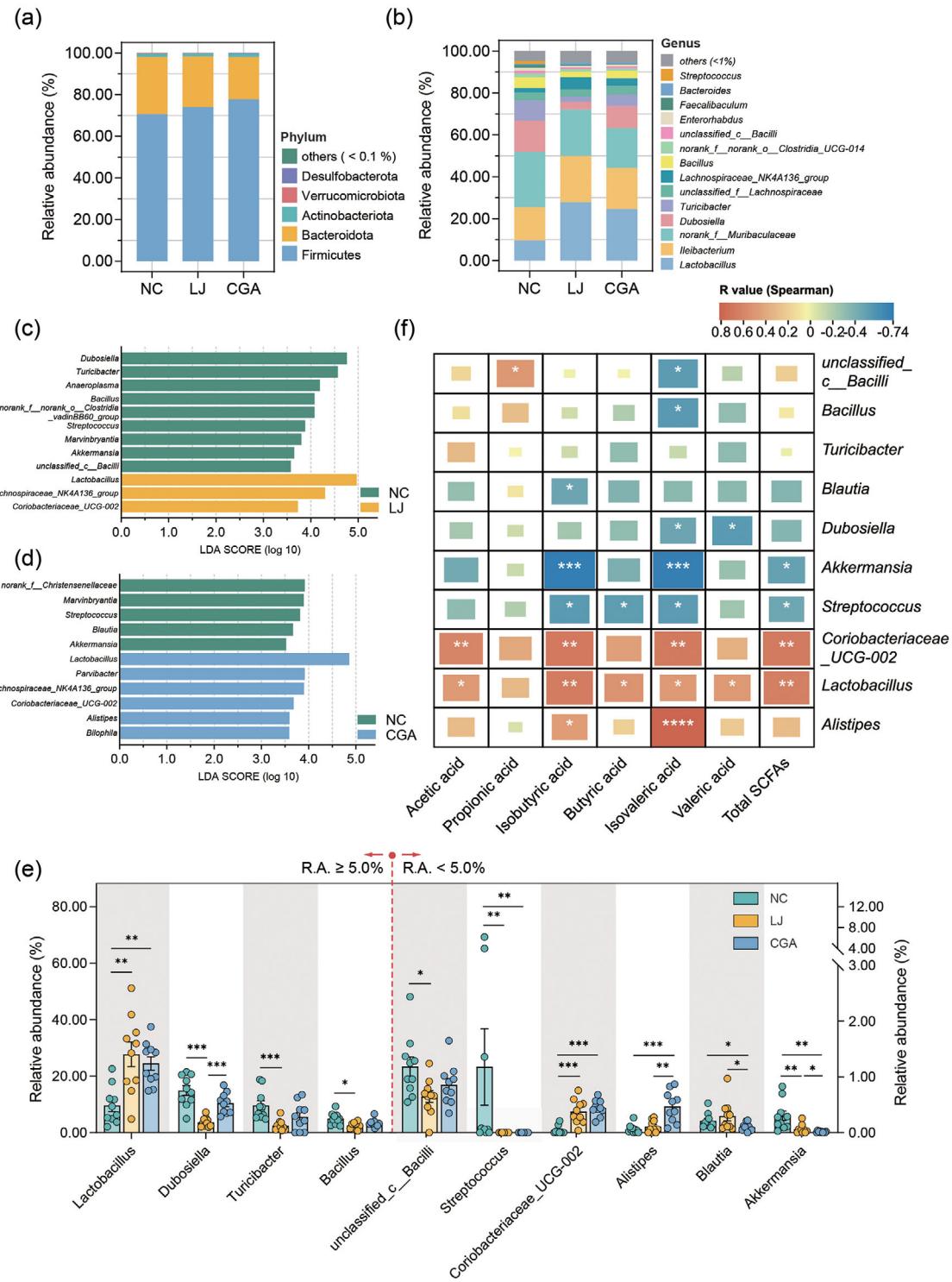
### 3.5 | LJ extract and CGA alleviated DSS-induced colitis in mice

Our previous study has demonstrated the potential anti-inflammatory and antioxidant effects of LJ extract and CGA in healthy mice. In this section, we further elucidated the effects by a murine colitis model. An experimental colitis model was established by administrating 3.5% DSS in water for 7 days (Figure 4a). At the end of the experiment, mice subjected to DSS exhibited marked hallmarks of colitis, including body weight loss, severe pathological symptoms as reflected by the DAI, and considerable colon shortening, which suggested that the colitis was successfully induced. Administration of LJ extract and CGA significantly attenuated DSS-induced colitis as evidenced by significantly relieved body weight loss (from Day 4), DAI, and colon shortening (Figure 4b–e). Histological analysis was performed to visualize DSS-induced colon tissue injury. As exhibited in Figure 4f, the healthy colon tissue in the CON group was intact and well-structured. Mucosa comprised well-arrayed crypts populated by a high proportion of goblet cells with a flat surface epithelium. In the DSS group, the mucosal architecture was badly damaged. The mucosa was much thinner and completely devoid of crypts and goblet cells. The surface epithelium was impaired to make erosion. In the submucosa, the tissue showed obvious edema with diffuse inflammatory cell infiltrates. In the LJ extract and CGA intervention group, the architecture of crypts was slightly altered with visible loss of goblet cells, and inflammatory cells were focally aggregated in the mucosa. Therefore, the injury of the colon was greatly alleviated by LJ extract and CGA administration.

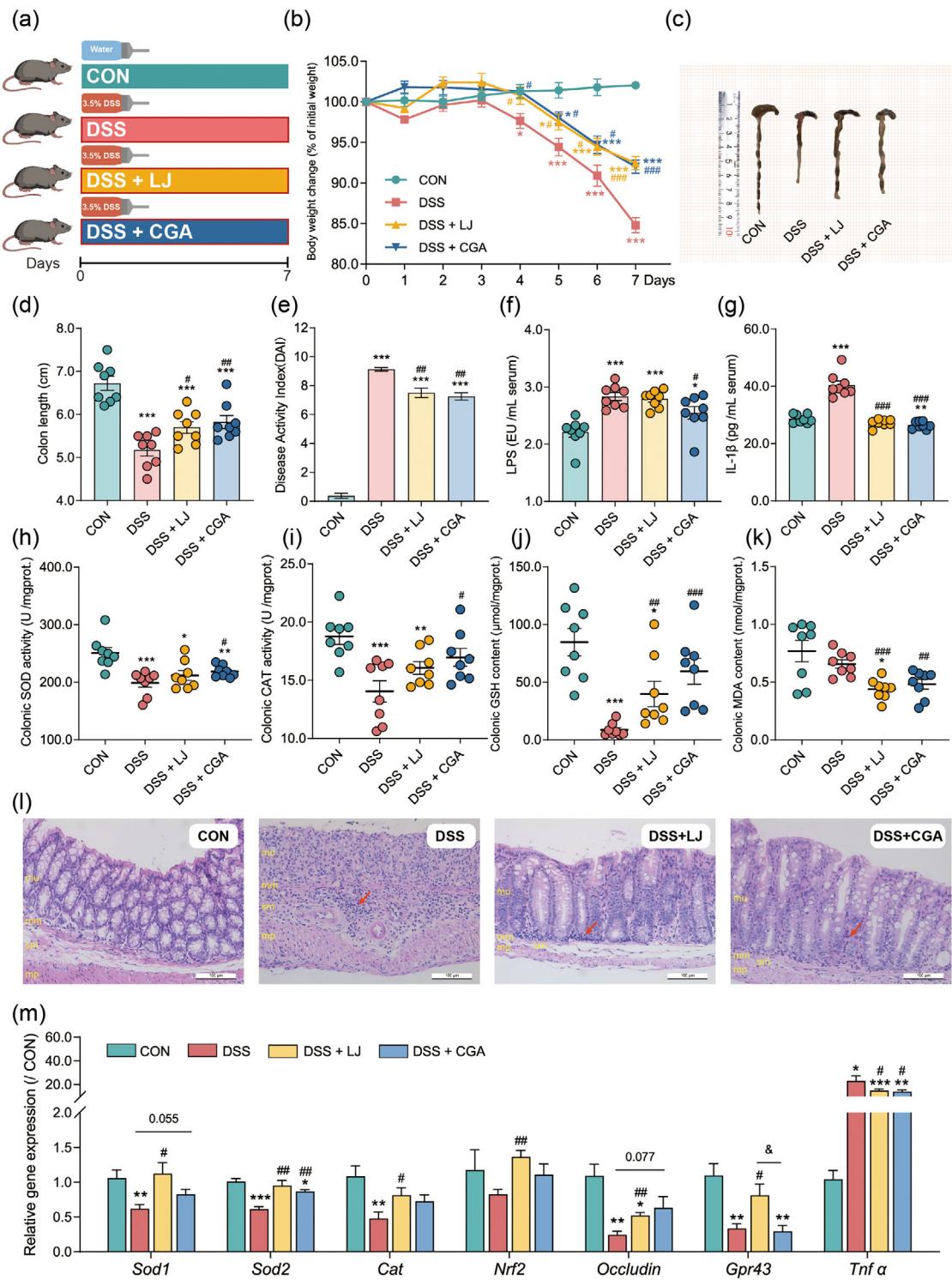
Defect of intestinal barrier integrity increased gut permeability, leading to the leakage of LPS into the systemic circulation and triggering inflammatory responses. The serum LPS concentration was measured to assess the integrity of the intestinal barrier. As shown in Figure 4f, the serum LPS concentrations of DSS-administrated mice were significantly higher than those of healthy mice, indicating the destruction of intestinal barrier integrity. Nevertheless, the serum LPS level was significantly decreased with CGA intervention, suggesting that CGA intake could alleviate the impairment of intestinal barrier function to some extent. Pro-inflammatory cytokines play a crucial role in driving colitis. We next measured serum IL-1 $\beta$  levels to evaluate the degree of inflammatory response. As displayed in Figure 4g, DSS treatment drastically elevated the serum IL-1 $\beta$  levels, while LJ extract and CGA administration significantly dampened this tendency, suggesting the anti-inflammatory effect of LJ extract and CGA.



**FIGURE 2** LJ and CGA led to a significant alteration of the gut microbiota composition. (a) Principal coordinate analysis (PCoA) plot of the gut microbiota composition at the amplicon sequence variant (ASV) level. Difference analysis was performed by ANOSIM. (b) Hierarchical clustering analysis at the ASV level. Alpha diversity of the gut microbiota represented by the Sobs index (c) and Shannon index (d) at the ASV level. One-way ANOVA with Tukey's multiple comparisons was used for multi-group comparisons. Independent samples' Student's t-test (for parametric datasets) or the Mann–Whitney U test (for non-parametric datasets) was used for the two groups' comparison. (e–g) Gut microbiota co-occurrence network analysis based on core genera. Red line indicates a positive Spearman's rank correlation, green line indicates a negative correlation ( $|r| > .6$ ,  $p < .05$ ). Thickness of the line represents the absolute value of the coefficient.



**FIGURE 3** LJ and CGA positively modulated the gut microbiota. Bacterial taxonomic profiling at the phylum level (a) and genus level (b) of gut microbiota. Linear discriminant analysis (LDA) effect size (LEfSe) analysis for differential abundant taxa detected between the normal control group (NC group) and LJ extract group (LJ group) (c) and CGA group (d) at the genus level. LDA score was > 3.0. (e) Comparison of the top 10 discriminative genera. (f) Spearman correlation analysis between the discriminative genera and fecal SCFAs profiles in healthy mice. One-way ANOVA with Tukey's multiple comparisons was used for multi-group comparisons. Independent samples' Student's t-test (for parametric datasets) or the Mann–Whitney U test (for non-parametric datasets) was used for the two groups' comparison. \* $p < .05$ , \*\* $p < .01$ , \*\*\* $p < .001$ , \*\*\*\* $p < .0001$ .



**FIGURE 4** LJ extract and CGA alleviated dextran sulfate sodium (DSS)-induced colitis in mice. **(a)** Study design for Animal Experiment 2. **(b)** Daily body weight changes during Animal Experiment 2. Representative macroscopic photos **(c)** and length of colons **(d)** of each group. **(e)** DAI of each group at the end of the experiment. Levels of serum LPS **(f)** and serum IL-1 $\beta$  **(g)**. Levels of colonic superoxide dismutase (SOD) activity **(h)**, CAT activity **(i)**, GSH level **(j)**, and malondialdehyde (MDA) level **(k)**. **(l)** Representative hematoxylin and eosin (H&E)-stained colon sections of each group (scale bar, 100  $\mu$ m. mu: mucosa, mm: muscularis mucosa, sm: submucosa, mp: muscularis propria. Red arrow indicates inflammatory cells infiltration). **(m)** Gene expression profiles of colon tissue involved in oxidative stress defense, inflammation, and tight junction. One-way ANOVA with Tukey's multiple comparisons was used for multi-group comparisons. Independent samples' Student's t-test (for parametric datasets) or the Mann–Whitney U test (for non-parametric datasets) was used for two groups' comparison. \* compared with CON group, \* $p < .05$ , \*\* $p < .01$ , \*\*\* $p < .001$ . # compared with DSS group, # $p < .05$ , ## $p < .01$ , ### $p < .001$ . &, comparison between treatment groups. & $p < .05$ .

Inflammation is intricately linked with oxidative stress. To further evaluate the colonic antioxidant status, we measured the colonic SOD and CAT activities and GSH and MDA content to assess the colonic antioxidant status. As exhibited in Figure 4h–k, DSS administration resulted in a significant decrease of colonic SOD, CAT activity, and GSH level, while LJ extract or CGA treatment greatly reversed the tendency. Concomitantly, colonic MDA production was also significantly suppressed by our treatment. These data suggested that LJ extract and CGA could restore the oxidative stress induced by DSS.

Having confirmed the anti-inflammatory and antioxidant properties of LJ extract and CGA, we further explored the underlying mechanisms. We evaluated the gene expressions involved in oxidative stress defense (*Sod1*, *Sod2*, *Cat*, *Nrf2*), inflammation (*Tnfα*) and tight junction (*Occludin*) at the transcriptional level in colon tissue. As displayed in Figure 4m, expressions of genes encoding antioxidant enzymes (*Sod1*, *Sod2*, *Cat*) and tight junction proteins (*Occludin*) were notably suppressed in the DSS group. However, intervention with LJ extract or CGA significantly reversed this trend. It was worth mentioning that LJ extract administration significantly elevated the expression of *Nrf2*, a major regulator of endogenous antioxidant pathways. Additionally, DSS administration strikingly promoted the expression of *Tnfα*, which could be significantly reversed by the intervention of LJ extract and CGA.

Collectively, these data elucidated that LJ extract and CGA intervention showed considerable relieving effects on DSS-induced murine colitis, and the effects were partially attributed to their great antioxidant and anti-inflammatory properties.

### 3.6 | LJ extract and CGA modulated the fecal SCFAs production and gut microbiota composition in DSS-induced colitis mice

SCFAs are increasingly favored by researchers for their excellent anti-inflammatory properties, with SCFA levels reported to be closely linked to the development of IBD. The changes of fecal SCFAs acid content after the intervention of DSS, LJ extract, and CGA are shown in Figure 5m. Only three SCFAs (acetic acid, propionic acid, and butyric acid) could be detected in DSS-administrated groups. As anticipated, the production of propionic acid and butyric acid was significantly diminished by DSS treatment, while the intervention of LJ extract and CGA could notably revitalize the production of all three SCFAs. G-protein coupled receptor 43 (*Gpr43*) recognizes SCFAs and is implicated in colitis (Ang et al., 2015). We measured the gene expression of *Gpr43* in colon tissue. Accordantly, *Gpr43* expression was highly suppressed by DSS, whereas LJ extract significantly reversed the tendency (Figure 4m).

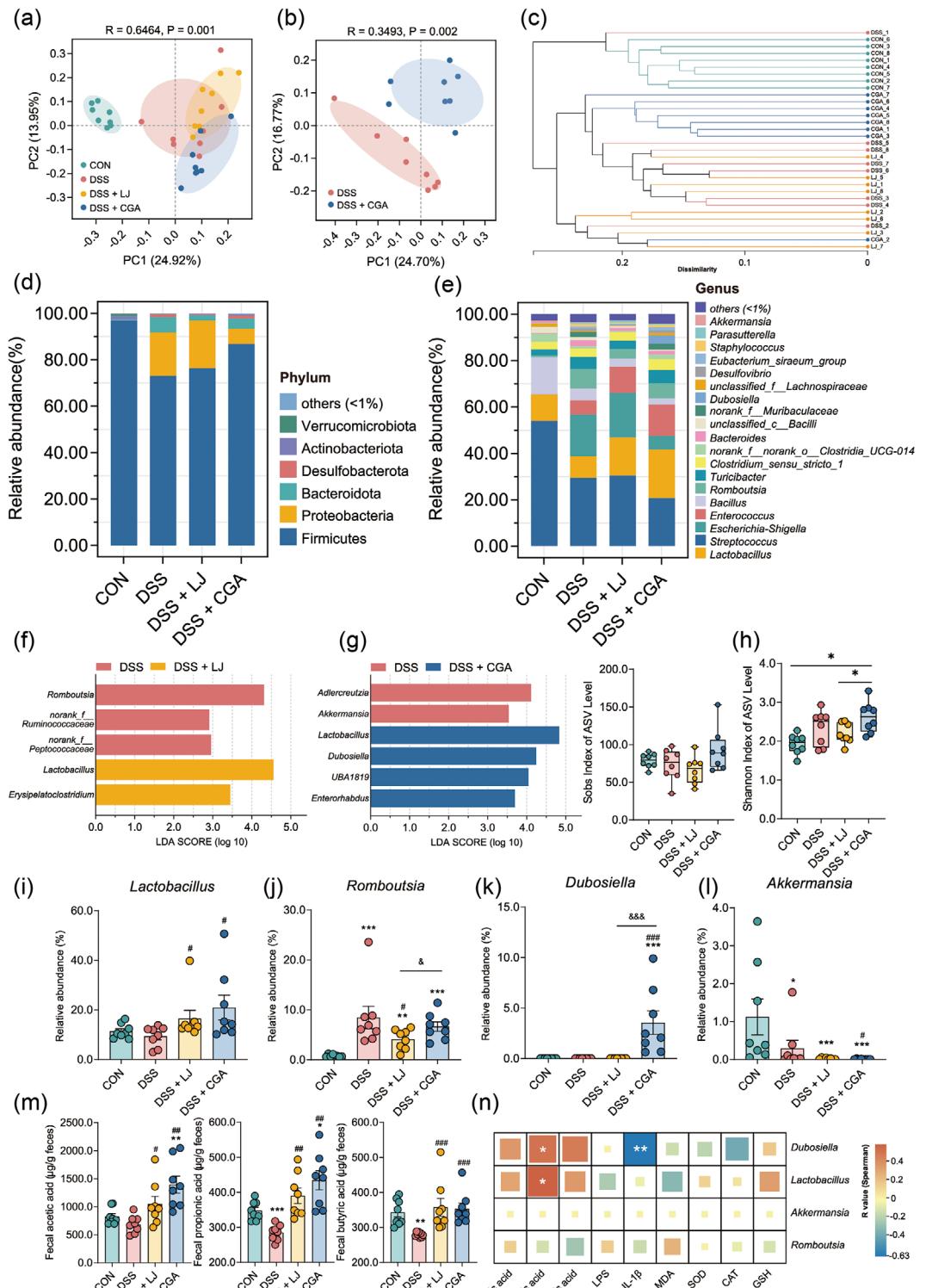
IBD is typically associated with gut microbiota dysbiosis. Given the positive modulatory effect of LJ extract and CGA, we further investigated whether LJ extract and CGA could protect against DSS-induced gut microbiota dysbiosis by 16S rRNA gene sequencing. A total of 350 ASVs were detected. Likewise, PCoA was first applied to distinguish the gut microbiota of the four groups. As portrayed in Figure 5a, DSS administration resulted in a substantial alteration

in the gut microbiota composition (ANOSIM:  $r = .6464$ ,  $p = .001$ ), indicating the gut microbiota dysbiosis induced by DSS. More specifically, we further conducted a two-group comparison between the DSS group and the LJ extract or CGA intervention group, and the results suggested that CGA intervention significantly impacted the gut bacterial composition (ANOSIM:  $r = .3493$ ,  $p = .002$ ; Figure 5b). Consistently, similar conclusion could be drawn in the hierarchical clustering analysis (Figure 5c). Alpha diversity analysis, including the Sobs and Shannon indices, revealed that neither DSS nor LJ extract administration exerted significant influence on the gut microbiota diversity, whereas CGA intervention could significantly elevate the diversity of the gut microbiota, compared with the CON group (Figure 5h). To further compare the overall composition of different groups, we portrayed the bacterial taxonomic profile at the phylum and genus levels (Figure 5d–e). Obviously, DSS administration tremendously decreased the proportion of Firmicutes and increased the proportion of Proteobacteria at the phylum level. While at the genus level, the average relative abundance of *Escherichia-Shigella*, *Enterococcus*, and *Romboutsia* was increased and that of *Streptococcus* and *Bacillus* was decreased in DSS-administrated groups.

We next worked to identify the possible key bacteria contributing to the mitigating effect of LJ extract and CGA on colitis by LEfSe analysis at the genus level. A total of five (DSS vs. DSS + LJ) and six (DSS vs. DSS + CGA) key discriminative genera were identified (Figure 5f–g). A detailed comparison of representative bacteria is displayed in Figure 5i–l. Interestingly, despite that DSS administration had no impact on the relative abundance of *Lactobacillus*, the intervention of both LJ extract and CGA significantly elevated the proportion of it. Notably, the growth of *Akkermansia* was substantially suppressed by DSS administration. The intervention of LJ extract specifically reversed the elevation of *Romboutsia* caused by DSS, and the intervention of CGA led to a dramatic enrichment of *Dubosiella*. As inspired by our previous results, we also performed Spearman correlation analysis between the key genera and fecal SCFAs profiles and antioxidant/inflammatory related parameters. As depicted in Figure 5n, there was a positive correlation between fecal SCFAs content and *Lactobacillus* and *Dubosiella*, while only the relationship with propionic acid reached a significant correlation. *Lactobacillus* was negatively correlated with serum LPS level ( $r = -.24$ ), colonic MDA level ( $r = -.34$ ) and positively correlated with colonic GSH level ( $r = .33$ ), although the relationship was not significant. Additionally, there was a significant negative correlation between *Dubosiella* and serum IL-1 $\beta$  level. No significant correlation was found referring to *Akkermansia* and *Romboutsia*.

### 3.7 | FMT of LJ and CGA-mediated gut microbiota alleviated DSS-induced colitis in mice

FMT is one of the most commonly applied approaches to investigate the causality between gut microbiota and diseases (Bokoliya et al., 2021). To this end, we next performed FMT to elucidate whether gut microbiota played a predominant role in the alleviating effects of LJ



**FIGURE 5** LJ extract and CGA modulated the gut microbiota composition and fecal SCFAs production in DSS-induced colitis mice. (a–b) PCoA plot of the gut microbiota composition at the ASV level. Difference analysis was performed by ANOSIM. (c) Hierarchical clustering analysis at the ASV level. Bacterial taxonomic profiling at the phylum level (d) and genus level (e) of gut microbiota. LEfSe analysis for differential abundant taxa detected between the DSS group and DSS + LJ group (f) and DSS + CGA group (g). (h) Alpha diversity of the gut microbiota is represented by the Sobs index and Shannon index. (i–l) Comparison of the representative discriminative genera. (m) Levels of fecal SCFAs production in colitis mice, including acetic acid, propionic acid, and butyric acid. (n) Spearman correlation analysis between the discriminative genera and fecal SCFAs profiles and antioxidant/inflammatory related parameters in colitis mice. One-way ANOVA with Tukey's multiple comparisons was used for multi-group comparisons. Independent samples' Student's t-test (for parametric datasets) or the Mann–Whitney U test (for non-parametric datasets) was used for the two groups' comparison. \* compared with CON group,  $*p < .05$ ,  $**p < .01$ ,  $***p < .001$ . # compared with DSS group,  $#p < .05$ ,  $##p < .01$ ,  $###p < .001$ . &, comparison between treatment groups. &  $p < .05$ .

extract and CGA on DSS-induced colitis. Fecal microbiota derived from NC, LJ, and CGA groups in Animal Experiment 1 were transplanted to DSS-administrated mice by gavage. As inspired by W.-L. Sun et al. (2021), we also set a placebo group for each FMT group as a control. In detail, fresh fecal suspensions of each FMT group were heat-treated to inactivate the gut microbiota and to obtain SFS. Both the SFS and FMT had the same fecal matter composition, differing only in whether the gut microbiota was alive. The duration of the experiment was 6 days, and the detailed procedure is illustrated in Figure 6a.

Body weight changes of the mice were monitored daily throughout the whole experiment. As exhibited in Figure 6b,c, no significant differences in the body weight changes were observed among groups in the first 2 days. However, starting from the third day, mice body weight loss in the LJ + FMT group and CGA + FMT group was significantly relieved, compared to the NC + FMT group. No significance in body weight loss was discovered between the NC + FMT group and the NC + SFS group except on Day 3. Interestingly, it seems that the relieving effect of SFS groups on body weight loss dissipated gradually. There are no significant differences in body weight loss between FMT groups of LJ and CGA and corresponding SFS groups before Day 5. However, starting from Day 5, the body weight loss of the LJ + SFS and CGA + SFS groups was notably higher than that of the corresponding FMT group. At the end of the experiment, we assessed the DAI and measured the colon length of mice in each group. As depicted in Figure 6d-f, the DAI of the LJ + FMT and CGA + FMT groups was significantly lower than that of the NC + FMT group and corresponding SFS group. Consistently, the colon length of the LJ + FMT and CGA + FMT groups was significantly longer than that of the NC + FMT group and corresponding SFS group. Histological evaluation was further conducted to assess the severity of colon damage. As shown in Figure 6g, the mucosal architecture in NC + FMT, NC + SFS, LJ + SFS, and CGA + SFS was badly impaired. Observed injuries included loss of crypts and goblet cells, cryptitis, surface epithelium erosion, submucosa edema, and diffuse inflammatory cells infiltration. However, these symptoms were greatly attenuated in the LJ + FMT and CGA + FMT groups. Taken together, these data suggested that the disease symptoms of colitis were relatively mild in the LJ + FMT and CGA + FMT, indicating that FMT of LJ and CGA-mediated gut microbiota could alleviate DSS-induced colitis in mice.

#### 4 | DISCUSSION

IBD has emerged as a global health challenge over the past decades, which poses substantial social and economic burden on governments and health systems (Alatab et al., 2020). Currently, applied drugs including aminosalicylates and immunomodulators are generally accompanied by systemic side effects and poor patient compliance (Zhou et al., 2022). Hence, there is a growing focus on natural products as alternative agents due to their safety and wide spectrum of bioactivities. LJ has a long history of extensive use in traditional Chinese medicine (TCM) and other Asian countries (Shang et al., 2011). The bud and flower of LJ is also an item of “medicine food homology” in China, an ancient concept that means some foods themselves are drugs, and

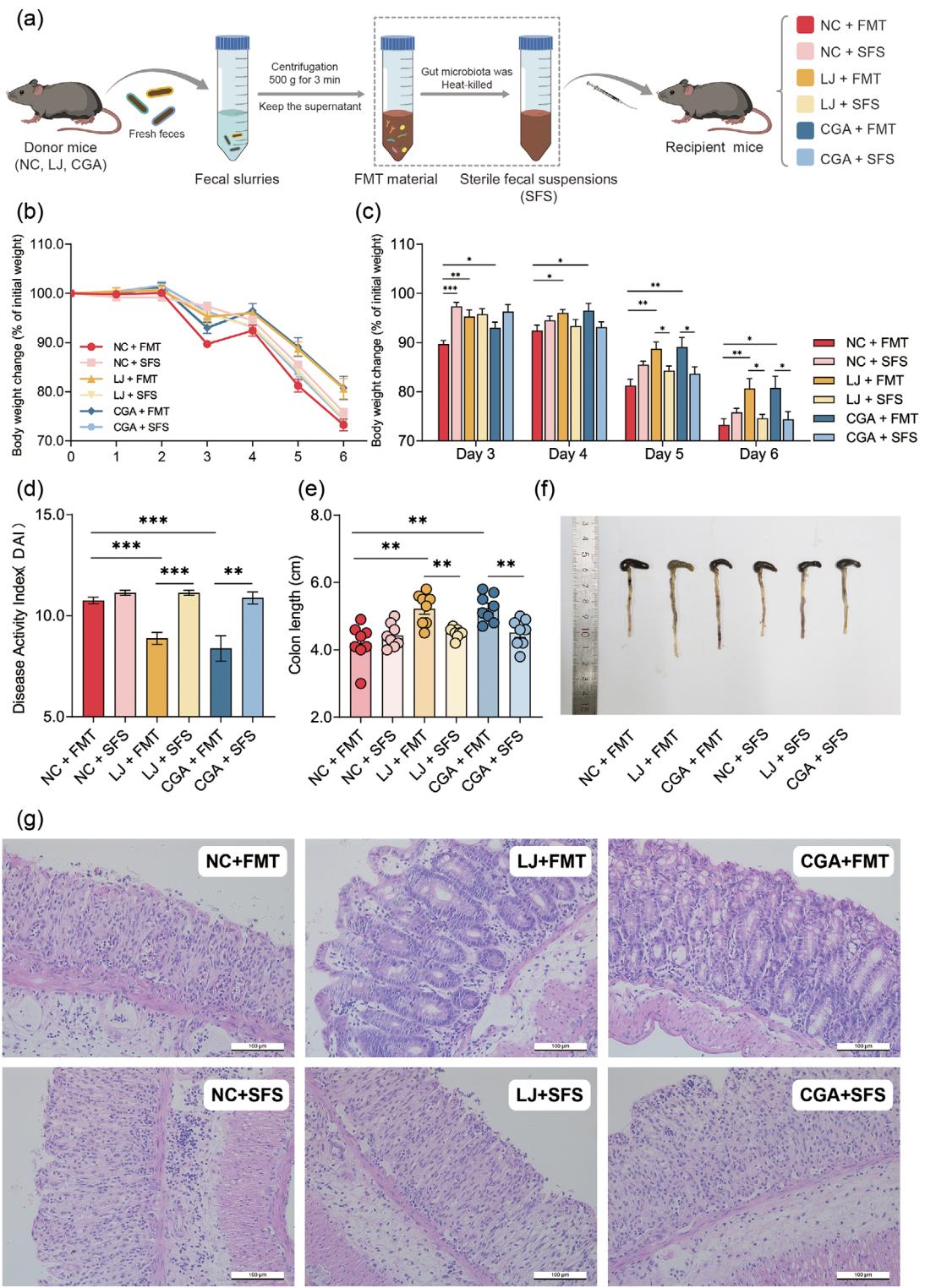
there is no absolute boundary between them, which indicates the great potential of LJ as modern functional foods (Hou & Jiang, 2013). In TCM clinical practice, LJ is recorded to have heat-clearing and detoxifying effects, often used to treat infectious diseases. These properties were embodied by modern pharmacological studies that suggest LJ as a safe and mild anti-inflammatory agent (Shang et al., 2011).

As mentioned above, LJ could be consumed as either FOOD or MEDICINE. We first investigated the effects of consuming LJ extract and CGA as FOOD by healthy objects. Healthy mice were daily oral administrated with LJ extract and CGA for 3 weeks. In this experiment, LJ extract and CGA were regarded as a simulation of dietary supplements for healthy individuals. As demonstrated by our results, the intervention of LJ extract and CGA has no obvious influence on the body weight and histology of the vital organs. These data suggested that oral administration of LJ extract and CGA in the present dose and duration is safe and exerted no observed side effects on the mice, which laid a solid foundation for subsequent experiments.

Subsequently, we paid close attention to the effects of LJ extract and CGA on the overall antioxidant status of healthy objects. Oxidative stress is responsible for decreased biological activities, immune overactivation, and inflammation (Diaz de Barboza et al., 2017) and is closely related with the onset/progression of several diseases such as cancer, diabetes, and IBD (Pizzino et al., 2017). To counterbalance oxidative stress, intricate antioxidant systems come into play, consisting of enzymatic defenses such as SOD and CAT; and non-enzymatic antioxidants including GSH and vitamins C and E (Ma, 2013). However, the endogenous antioxidant systems are sometimes not enough to prevent oxidative damage, and exogenous antioxidant additives are employed to delay or prevent oxidation. Natural products have emerged as promising candidates for antioxidant properties.

Several in vitro and in vivo studies have demonstrated that LJ and CGA showed potent protective effects against oxidative stress either by promoting enzymatic defense (SOD and CAT activities) or by elevating non-enzymatic antioxidants (GSH) while reducing the marker of oxidative injury (MDA) (Gu et al., 2023; Luo et al., 2019; F. Wang et al., 2017; Y. Zhang et al., 2018). However, most of the studies were conducted under pathological condition (e.g., diabetes), with limited exploration into the effect of LJ or CGA on the antioxidant status of healthy objects. In our study, we found that intervention of LJ extract or CGA significantly promoted serum CAT activity, GSH content and colonic AOC. These data suggested that LJ and CGA showed a significant effect on maintaining the antioxidant state of healthy body.

The modulation effect of LJ extract and CGA on healthy gut microbiota is another focus of interest in the experiment. Gut microbiota plays a crucial role in many aspects of human health including immune, metabolic, and neurobehavioral traits (Valdes et al., 2018). A growing body of evidences has suggested that the health benefits of polyphenols may partially rely on the modulation of the gut microbiota (Anhê et al., 2019). Diet serves as a key environmental factor in initiating, shaping, and modulating the gut microbiota (Loo et al., 2020). Accordingly, modulation of the gut microbiota by diet is believed to be a promising and effective way to benefit the host (Han & Xiao, 2020). To the best of our knowledge, we are the first to explore the



**FIGURE 6** Fecal microbiota transplantation (FMT) of LJ and CGA-mediated gut microbiota alleviated DSS-induced colitis in mice. (a) Study design for Animal Experiment 3. (b–c) Daily body weight changes during Animal Experiment 3. (d) DAI of each group at the end of the experiment. Representative macroscopic photos (f) and length of colons (e) of each group. (g) Representative H&E-stained colon sections of each group (Scale bar, 100  $\mu$ m). One-way ANOVA with Tukey's multiple comparisons was used for multi-group comparisons. Independent samples' Student's t-test (for parametric datasets) or the Mann–Whitney U test (for non-parametric datasets) was used for the two groups' comparison. \* $p < .05$ , \*\* $p < .01$ , \*\*\* $p < .001$ .

effects of LJ extract and CGA on the gut microbiota of healthy individuals. To our expectation, long-term intervention of LJ extract and CGA led to a dramatic alteration of the gut microbiota structure as revealed by PCoA and hierarchical clustering analysis. And the gut microbiota profiles of the LJ group and CGA group exhibited substantial similarity. Despite the fact that LJ and CGA had no obvious impact on the diversity of the bacterial community, the subsequently conducted co-occurrence network indicated that consumption of LJ extract enhanced the complexity and stability of the gut microbiota.

Notably, the intervention of both LJ extract and CGA contributed to a dramatic elevation of *Lactobacillus*. *Lactobacillus* is a genus of rod-shaped, gram-positive, non-spore-forming, and facultatively anaerobic bacteria of the phylum Firmicutes (Dempsey & Corr, 2022). Strains of *Lactobacillus* have a long history of safe and effective use as probiotics due to their perceived health-promoting effects (Sanders et al., 2019). Lactate is the primary end product of *Lactobacillus* fermentation. Although lactate is not an SCFA, it could be utilized by certain bacteria to produce SCFAs (acetate, propionate, and butyrate) through bacterial cross-feeding (Flint et al., 2015; Ríos-Covián et al., 2016). Evidences have supported that *Lactobacillus* strains consumed as probiotics promote gastrointestinal health via multiple mechanisms, including increasing SCFAs and mucus production, enhancing tight junction integrity, competing against pathogens, and so forth (Dempsey et al., 2022). Previous studies have also demonstrated that polyphenols present prebiotic effect through favoring the growth of *Lactobacillus* and increasing the production of SCFAs (Alves-Santos et al., 2020). On the other hand, the intervention of LJ extract and CGA both decreased the relative abundance of *Akkermansia*. Nowadays, numerous studies have proven the efficacy of *Akkermansia* to improve obesity, diabetes mellitus, hepatic steatosis, intestinal inflammation, and different cancers, which has repeatedly emerged as a promising candidate for next-generation probiotics (Cani et al., 2022). In most cases, CGA is reported to upregulate *Akkermansia* under morbid conditions such as obesity (X. Gao et al., 2024) and colitis (Z. Zhang et al., 2017), while few studies investigated the effect of CGA on *Akkermansia* under healthy status. However, Gao et al. found that CGA did not directly promote *Akkermansia muciniphila* growth or mucin secretion but through the inhibition of *Desulfovibrio* and *Alistipes* and the influence of fecal metabolites. This phenomenon indicated that alteration of the microbiota is also dependent on the microbes-mediated interactive action (X. Gao et al., 2024). This clue made our results rational, as the intervention of LJ extract and CGA might create a bacterial environment that unfavored the growth of *Akkermansia*. Other factors such as the health status of the host, mice diet, administration doses, and experiment duration might also account for the results. Additionally, the intervention of LJ extract or CGA specifically impacted the abundance of other bacteria such as *Coriobacteriaceae\_UCG-002*, *Streptococcus*, *Dubosiella*, *Turicibacter*, *Bacillus*, *Alistipes*, and *Blautia*. Nevertheless, evaluations of these genera were quite controversial. This phenomenon indicated that the complex interactions existing within the microbiome ecosystems and the presence of potential confounding factors require taking into consideration when concerned about the effect of a single symbiont (Cani et al., 2022).

As described above, the promotion of *Lactobacillus* by LJ extract and CGA may lead to an elevation in SCFAs production. We further validated the hypothesis by measuring the fecal SCFAs levels. As expected, the intervention of LJ and CGA dramatically increased the production of SCFAs including acetic acid, propionic acid, butyric acid, isobutyric acid, and isovaleric acid. Subsequent correlation analysis indicated that *Lactobacillus* and *Coriobacteriaceae\_UCG-002* were positively correlated with the content of most SCFAs ( $p < .05$ ), and *Alistipes* was positively correlated with isobutyric acid and isovaleric acid content ( $p < .05$ ). SCFAs are now considered as key mediators in the diet–microbiota–host crosstalk. Mounting evidence has highlighted the multifaceted beneficial effects of SCFAs on host health. SCFAs not only exert multiple benefits locally to improve gut health, but also enter the systematic circulation and directly influence the metabolism or the function of tissues and organs beyond the gut (Canfora et al., 2015; Dalile et al., 2019; Morrison & Preston, 2016; van der Hee & Wells, 2021). Accordingly, LJ extract and CGA remarkably promoted the growth of *Lactobacillus* and other potential SCFAs-producing bacteria, which subsequently boosted the SCFAs production. It is very likely that the benefits induced by elevated *Lactobacillus* and SCFAs could compensate the potential shortcoming, which may result from the decrease of *Akkermansia*, thus exerting health-promoting effects on healthy individuals.

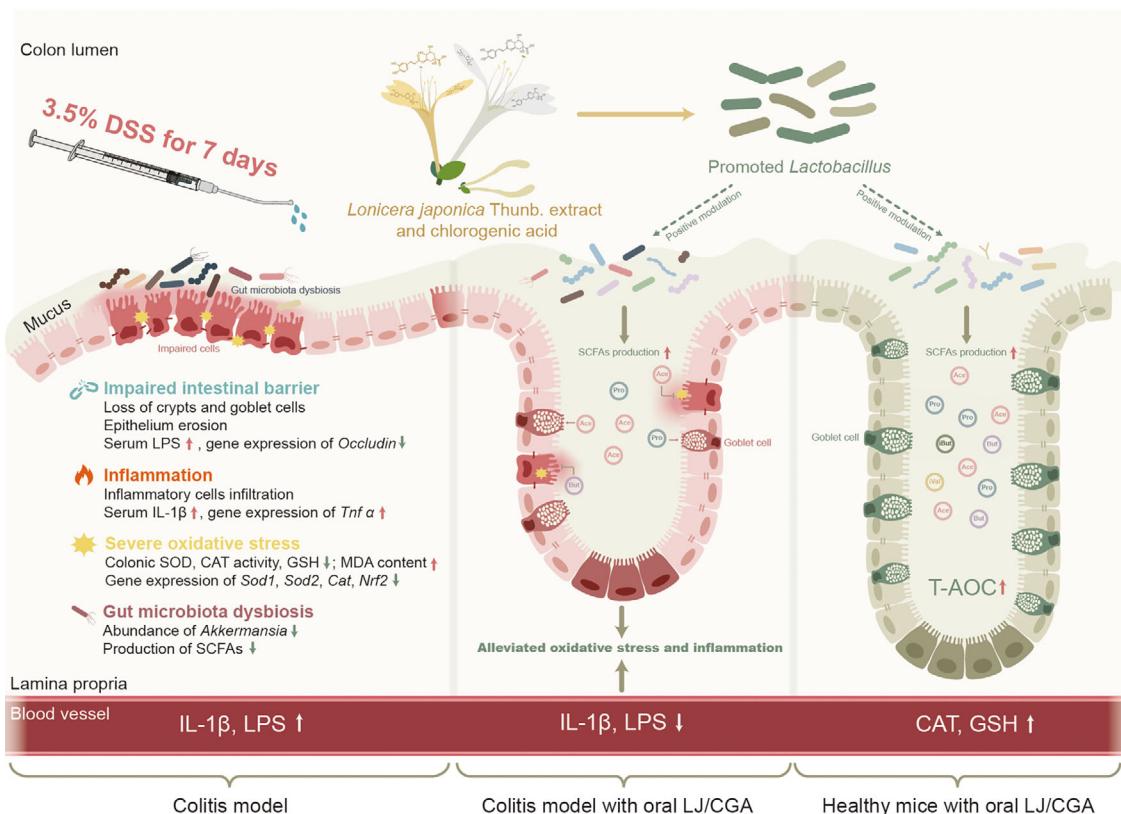
Based on the above findings, we next sought to investigate the alleviating effects of consuming LJ extract and CGA as MEDICINE on colitis. The in vitro anti-inflammatory effect of LJ extract was verified with an LPS-induced RAW267.7 cell model. To explore the alleviating effects of LJ extract and CGA on colitis, acute murine colitis was induced by 3.5% DSS, followed by oral administration with LJ extract and CGA for 7 days. It has been reported that consumption of LJ extract and CGA did have therapeutic effects on murine colitis (W. Gao et al., 2019; D. Liu et al., 2020; Park et al., 2013; Shin et al., 2015; Wan et al., 2021). In agreement with previous studies, in our study, the intervention of LJ extract and CGA significantly relieved colitis symptoms as reflected by decreased DAI and histological injury of the colon. In addition, oral administration of LJ extract and CGA attenuated DSS-induced inflammation as evidenced by decreased levels of serum inflammatory cytokine IL-1 $\beta$  and *Tnf*  $\alpha$  gene expression. Moreover, LJ extract and CGA intervention also maintained intestinal integrity as exemplified by decreased level of serum LPS and increased expression of tight junction *Occludin*. Intriguingly, oral administration of LJ and CGA fairly restored the antioxidant defense by increasing colonic SOD, CAT activity, and GSH content and decreasing the level of MDA, which may be attributed to the upregulation of related genes such as *Sod1*, *Sod2*, *Cat*, and *Nrf2*. The nuclear factor erythroid 2-related factor 2 (Nrf2) is a vital regulator of cellular resistance to oxidative stress. Nrf2 modulates the basal and induced expression of an array of antioxidant response element-dependent genes to respond to the physiological and pathophysiological outcomes of oxidative stress (Ma, 2013). These data validated that oral LJ extract and CGA showed potent relieving effects on DSS-induced colon inflammation and oxidative stress.

Several studies have proposed that natural products could alleviate murine colitis by modulating the gut microbiota. The investigated natural products were of great diversity, such as epigallocatechin-3-gallate

from green tea (Wu et al., 2021), acteoside from *Osmanthus fragrans* flowers (Y. Liu, Huang, Zhu, et al., 2022), rutin from *Sophora japonica* flowers (Y. Liu, Huang, Ji, et al., 2022), and so forth. Inspired by these findings, we hypothesized that the therapeutic effects of LJ extract and CGA might be derived from their modulation on gut microbiota and its metabolism. In line with previous studies, DSS administration caused an evident alteration of the gut microbiota structure in our study, which is usually referred to as dysbiosis (D. Li et al., 2021; Y. Liu, Huang, Ji, et al., 2022; Y. Liu, Huang, Zhu, et al., 2022; Wu et al., 2021), whereas LJ extract and CGA intervention could reverse this trend to some extent. Generally, the most prominent changes in the gut microbiota of IBD patients are a reduction in microbial diversity associated with a decreased abundance of Firmicutes alongside an increased abundance of Proteobacteria (Guo et al., 2020; Quaglio et al., 2022). In the present study, although DSS administration had little impact on the gut microbiota diversity, it did lead to a significant decrease in Firmicutes and an increase in Proteobacteria at the phylum level, while CGA obviously reversed this tendency. Normally, *Akkermansia* was dramatically reduced in patients with IBD (Png et al., 2010; Rajilić-Stojanović et al., 2013). Indeed, DSS administration resulted in a notable decrease in the abundance of *Akkermansia* in the present study. Supplementation with *A. muciniphila* has been reported to ameliorate DSS-induced colitis in mice (Bian et al., 2019), and studies also demonstrated that natural products could alleviate colitis by promoting the growth of *Akkermansia* (Huang et al., 2021; F. Liu et al., 2021; Y. Liu, Huang, Zhu, et al., 2022; Wu et al., 2021). These data indicated that *Akkermansia* might play a vital role in protecting against intestinal injury. However, the intervention of LJ extract and CGA showed no impact on the abundance of *Akkermansia*, which suggested that the therapeutic effects of LJ extract and CGA on colitis were independent of *Akkermansia*. By contrast, oral administration of LJ extract and CGA apparently increased the abundance of *Lactobacillus*, which was in a like manner with their effects on healthy gut microbiota. *Lactobacillus* has been proven to alleviate colitis in both DSS-induced murine colitis and IBD patients as representative bacteria of probiotic-assisted therapy. The protective effects of *Lactobacillus* on IBD and underlying mechanisms have been clearly recapitulated (C. Li et al., 2023). SCFAs have profound effects on maintaining intestinal homeostasis, and studies have shown that fecal SCFAs levels were particularly impaired in active IBD (Parada Venegas et al., 2019; M. Sun et al., 2017). SCFAs have been considered as promising supplementary treatment in the current clinical management of active IBD patients and diversion colitis (Parada Venegas et al., 2019). Likewise, DSS-administration caused a significant depletion in the production of fecal propionic and butyric acid in the present study. In particular, the intervention of LJ extract and CGA remarkably promoted the production of acetic, propionic, and butyric acid. SCFAs are natural ligands for G-protein-coupled receptors (GPCRs) including GPR41, GPR43, and GPR109a. There are considerable evidences that SCFAs protect against intestinal inflammation through intestinal epithelial barrier maintenance and immune regulation by activating GPCRs (M. Sun et al., 2017). Here, we found that DSS administration significantly impaired the gene expression of colon *Gpr43*, while LJ extract could restore its expression. It is reported that *Gpr43*<sup>-/-</sup>

mice showed exacerbated or unresolved inflammation in models of colitis, which indicated that GPR43 binding of SCFAs potentially provides a molecular link between bacterial metabolism and immune and inflammatory responses (Maslowski et al., 2009). Taken together, we inferred that the therapeutic effects of LJ extract and CGA on colitis are dependent of the regulation of *Lactobacillus* and SCFAs rather than *Akkermansia*. The intervention of LJ extract and CGA favored the growth of *Lactobacillus* in colitis mice, followed by the promotion of the production of SCFAs, which subsequently alleviated DSS-induced inflammation and oxidative stress.

To validate the hypothesis that the gut microbiota and their metabolites played a key role in the therapeutic effects of LJ extract and CGA, we further performed FMT and SFS from normal controls and LJ or CGA-administered mice in the DSS-induced colitis model. Exhilaratingly, LJ + FMT and CGA + FMT evidently alleviated colitis symptoms, compared with the NC + FMT group, in a like manner of intervention of LJ and CGA themselves. LJ + FMT and CGA + FMT have higher proportions of *Lactobacillus* and SCFAs levels than that of NC + FMT. This preliminarily verified that LJ and CGA-mediated gut microbiota or their metabolites played an essential role in the therapeutic effects of LJ and CGA. Next, we sought to find out which of the two contributed more to the therapeutic effects, gut microbiota or their metabolites. We discovered that SFS groups of LJ and CGA relieved the colitis in the first days during the experiment as compared with the NC + FMT group. This finding suggested that the bacterial metabolites did have a positive effect in alleviating colitis. Nevertheless, the protective effects of SFS disappeared as the experiment proceeded. And the severity of colitis in LJ/CGA + SFS groups was significantly heavier than that of the LJ/CGA + FMT group at the end of the group. We speculated that metabolites including SCFAs in SFS could alleviate colitis when the symptoms were still mild. Unfortunately, as the experiment progressed, the colitis worsened, and the SCFAs were depleted and could not meet the need to fight against inflammation. By contrast, bacteria, especially *Lactobacillus* in FMT, could continuously promote the production of SCFAs and further protect against colitis. This assumption could be partially explained by the fact there was no obvious difference between the NC + FMT and NC + SFS groups in the colitis symptoms, as both two groups contained low levels of SCFAs. It was also supported by a previous study that demonstrated that administration of sterile fecal filtrate was not able to elevate the fecal SCFAs of recipient mice (Wu et al., 2021). Strangely, all SFS groups showed no obvious differences referring to colitis severity. This result may be attributed to the possibility that the concentration difference of SCFAs among the groups was not sufficient enough to cause an alteration on the alleviating effects. Collectively, these findings confirmed the hypothesis that the gut microbiota and SCFAs played an indispensable role in the therapeutic effects of LJ and CGA on DSS-induced colitis, and *Lactobacillus* is suggested to have a much more profound effect. Based on the existing results, we could also infer that the therapeutic effects of LJ on DSS-induced murine colitis were partially attributed to the CGA it contained. Nevertheless, there are thousands of chemical components in LJ extract, and the final pharmacological effects were derived from the synergistic or antagonistic interactions of the phytochemicals it con-



**FIGURE 7** Consumption of LJ extract and CGA benefits the host by positively modulating the gut microbiota and its metabolism. The intervention of LJ extract and CGA dramatically favored the growth of the important probiotic *Lactobacillus* and subsequently promoted the production of SCFAs both in healthy and colitis mice, which further alleviated inflammation, intestinal barrier injury, and oxidative stress induced by DSS.

tains. Therefore, the detailed mechanisms of action still require further investigation.

## 5 | CONCLUSION

In summary, the consumption of LJ extract and CGA benefits the host by enhancing the endogenous antioxidant system and positively modulating the gut microbiota. Notably, the intervention of LJ extract and CGA dramatically favored the growth of the important probiotic *Lactobacillus* and subsequently promoted the production of SCFAs in healthy individuals. In particular, LJ extract and CGA significantly alleviated inflammation, intestinal barrier injury and oxidative stress in DSS-induced murine colitis. The therapeutic effects were believed to be mediated by the promotion of *Lactobacillus* and SCFAs production, and the proposed mechanisms are summarized in Figure 7. Our findings should be of great value in considering consuming LJ and CGA as dietary supplementation or as novel therapies against IBD.

## AUTHOR CONTRIBUTIONS

**Jiebiao Chen:** Conceptualization; formal analysis; investigation; methodology; validation; visualization; writing—original draft. **Wan-**

**hua Xu:** Investigation; writing—original draft. **Yang Liu:** Investigation; methodology. **Xiao Liang:** Writing—original draft. **Yunyi Chen:** Investigation. **Jiaojiao Liang:** Investigation. **Jiping Cao:** Validation; writing—review and editing. **Baiyi Lu:** Writing—review and editing. **Chongde Sun:** Conceptualization; project administration; supervision. **Yue Wang:** Conceptualization; funding acquisition; project administration; supervision; writing—review and editing.

## ACKNOWLEDGMENTS

This work was supported by the National Natural Science Foundation of China (32101932), the Key Research and Development Program of Zhejiang Province (2021C02018), Fundamental Research Funds for the Central Universities (226-2022-00173, 226-2022-00215), and Ji'an Science and Technology Plan Project (20211-055350).

## CONFLICT OF INTEREST STATEMENT

The authors declare no potential conflicts of interest.

## ETHICS STATEMENT

All animal experiments were conducted in accordance with the Committee on the Ethics of Animal Experiments of Zhejiang University with the Permission Number ZJU20220372.

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

**How to cite this article:** Chen, J., Xu, W., Liu, Y., Liang, X., Chen, Y., Liang, J., Cao, J., Lu, B., Sun, C., & Wang, Y. (2024). *Lonicera japonica* Thunb. and its characteristic component chlorogenic acid alleviated experimental colitis by promoting *Lactobacillus* and fecal short-chain fatty acids production. *Food Frontiers*, 1–20. <https://doi.org/10.1002/fft2.412>