applied sciences

MDPI

Article

Phlorotannins Isolated from *Eisenia bicyclis* and *Lactobacillus casei* Ameliorate Dextran Sulfate Sodium-Induced Colitis in Mice through the AhR Pathway

Yeon Gyeong Go [1], Qunzhe Wang [1], Jumin Park [1], Hae-Jeung Lee [2,3,*,†] and Hyemee Kim [1,*,†]

[1] Department of Food Science and Nutrition & Kimchi Research Institute, Pusan National University, Busan 46241, Republic of Korea; yeongyeong0162@naver.com (Y.G.G.); 1939104850jyb@gmail.com (Q.W.); juminpark@pusan.ac.kr (J.P.)
[2] Department of Food and Nutrition & Institute for Aging and Clinical Nutrition Research, Gachon University, Seongnam-si 13120, Republic of Korea
[3] Department of Health Sciences and Technology, GAIHST, Gachon University, Incheon 21999, Republic of Korea
* Correspondence: skysea@gachon.ac.kr (H.-J.L.); kimhyemee@pusan.ac.kr (H.K.); Tel.: +82-31-750-5968 (H.-J.L.); +82-51-510-2847 (H.K.)
† These authors contributed equally to this work.

Abstract: Ulcerative colitis (UC), an inflammatory bowel disease (IBD) linked to colon cancer, needs effective natural preventive and therapeutic strategies to alleviate its clinical course. This study investigated the combined effects of phlorotannins (TAs) isolated from *Eisenia bicyclis* (*E. bicyclis*) and *Lactobacillus casei* (LC) on inflammatory markers in UC, with a focus on the aryl hydrocarbon receptor (AhR) axis. In vitro experiments revealed anti-inflammatory effects of the phlorotannin fraction isolated from *E. bicyclis*, especially in synergy with LC. In vivo experiments showed that a synbiotic combination of TAs and LC mitigated DSS-induced colitis and reduced intestinal shortening and splenic hypertrophy. The TA and LC combination suppressed inflammatory factors (IL-6, TNF-α, Lipocalin 2), while activating tight junction genes (Muc2, Zo-1, Occludin, and Claudin1) and enhancing antioxidant capacity (Nrf2 and Nqo1 genes). Activation of the AhR pathway, which is crucial for regulating intestinal inflammation via IL-22, was evident with both phlorotannin alone and synbiotic administration. The combination of TAs and LC amplified the synergistic effect on intestinal immunity and microbiota, favoring beneficial species and optimizing the Firmicutes/Bacteroidetes ratio. Overall, synbiotic use demonstrated superior preventive effects against UC, suggesting its potential benefits for improving the gut immune system through gut microbiota-derived metabolites.

Keywords: *Eisenia bicyclis*; *Lactobacillus casei*; colitis; anti-inflammatory; AhR pathway

Citation: Go, Y.G.; Wang, Q.; Park, J.; Lee, H.-J.; Kim, H. Phlorotannins Isolated from *Eisenia bicyclis* and *Lactobacillus casei* Ameliorate Dextran Sulfate Sodium-Induced Colitis in Mice through the AhR Pathway. *Appl. Sci.* **2024**, *14*, 2835. https://doi.org/10.3390/app14072835

Academic Editor: Maria Filomena Botelho

Received: 19 February 2024
Revised: 24 March 2024
Accepted: 26 March 2024
Published: 27 March 2024

1. Introduction

Ulcerative colitis (UC) is an inflammatory bowel disease (IBD) characterized by symptoms such as abdominal pain, diarrhea, rectal bleeding, and weight loss, with an increased risk of colorectal cancer [1]. Although the exact cause of UC remains unclear, it is generally considered a multifactorial condition triggered by genetic, environmental, and microbial factors, resulting in inflammation of the colonic mucosa, disruption of tight junctions, heightened production of pro-inflammatory cytokines, and induction of dysbiosis in the gut microbiota [2,3]. Cytokines act as signaling molecules in the inflammatory process and play a significant role in the onset and progression of inflammation and UC by activating inflammatory pathways and mediating the generation of inflammatory mediators [4]. Recognized as a global public health challenge by the World Health

Organization, the incidence of UC is surging, owing to shifts in dietary habits and heightened stress [5]. The existing medications for UC, including 5-aminosalicylates, systemic and local corticosteroids, and immunomodulators, may cause severe side effects [6]. Therefore, there is an urgent need for more effective natural preventive and therapeutic approaches to alleviate the clinical course of this disease.

Eisenia bicyclis (E.bicyclis), a brown marine algae, is rich in the polyphenol phlorotannin, consisting of phloroglucinol. Phlorotannins isolated from *E. bicyclis* include eckol, phlorofucofuroeckol-A, dieckol, and phloroglucional-A [7,8]. Extensive research has illuminated the diverse physiological activities of phlorotannins, including their antioxidant, anti-inflammatory, anti-allergic, neuroprotective, and memory-enhancing effects [9,10]. Furthermore, phlorotannins from *E. bicyclis* have demonstrated positive effects on tight junction cells, affecting the integrity of the intestinal mucosal barrier and contributing to the regulation of gut microbiota, thereby preventing and inhibiting the onset of UC [11]. In addition, phlorotannins, which are categorized as plant polymeric polyphenols, are considered prebiotic candidates because of their potential to interact with and stimulate the growth and metabolite production of the gut microbiota [12,13].

Probiotics, particularly various strains of Lactobacillus, such as *Lactobacillus casei* and *Lactobacillus reuteri*, have been reported to alleviate colitis in animal models by modulating the gut bacterial composition, decreasing inflammation-related cytokine production, and improving intestinal membrane function [14,15]. In addition, probiotics demonstrate synergistic effects when combined with polysaccharides or polymeric polyphenols, contributing to improved metabolic rates through digestion and improved gut microbiota composition [16]. The coexistence of specific bacteria with prebiotics, offering greater health benefits to the host than individual substances and producing synergistic effects via the production of bacterial metabolites, is referred to as synbiotics [17,18]. For example, the synergistic effects of *Ligilactobacillus salivarius* and the plant-derived prebiotic resveratrol have demonstrated efficacy in ameliorating colitis in mice through a reduction in inflammatory cytokine (IL-1β and IL-6) levels [19]. Previous findings have indicated that probiotic Lactobacillus can metabolize phlorotannins, producing beneficial metabolites [15,20]. However, limited research exists on whether synbiotics formed through the interaction of phlorotannins and probiotics have preventive and therapeutic effects on UC and their underlying mechanisms.

The aryl hydrocarbon receptor (AhR) is a transcription factor that is increasingly recognized for its pivotal role in intestinal immune regulation and tight junction modulation in UC [21,22]. Upon binding to diverse ligands, including xenobiotics, natural products, and microbiome metabolites, AhR undergoes cytoplasmic-to-nuclear translocation. In the nucleus, it regulates the expression of cytochromes (CYP1A1, CYP1B1), NQO1, and IL-22 mRNA, and preserves tight junctions and participates in the control of the intestinal immune response, including the suppression of NF-kB activity, the induction of the Nrf2 pathway, and the production of anti-inflammatory cytokines [23,24]. In recent years, many studies have shown that the metabolites of prebiotics produced by intestinal flora, such as polyphenols, tryptophan metabolites, and short-chain fatty acids (SCFAs), are considered natural AhR ligands. Phloroglucinol, a unique metabolite contained in brown algae, has also been identified as a ligand for AhR activity [25,26]. Considering that the expression of AhR is not only directly related to the distribution of intestinal flora, but also, the metabolites of prebiotics can regulate the activation of AhR; synbiotics that combine prebiotics and probiotics are considered to be potent potential regulators of AhR.

Although *E. bicyclis* has been reported to have antioxidant and anti-inflammatory activity, research concerning specific lactic acid bacteria that synergize with *E. bicyclis* and their effects on the AhR pathway via phlorotannin metabolites remains insufficient. This study aims to focus on the anti-inflammatory effects of phlorotannins isolated from *E. bicyclis* and exploring the symbiotic effects with the Lactobacillus strain *Lactobacillus casei* in a mice model of UC. We hypothesized that the combination of phlorotannins and *Lactobacillus casei* might mitigate colitis by modulating the AhR pathway.

2. Materials and Methods

2.1. Isolation of Phlorotannin-Rich Fractions from E. bicyclis

E. bicyclis was purchased from the Ulleungdo Mall (http://www.ulleungdomall.com/; 28 October 2022). The phlorotannin-rich fractions were extracted according to established methods [27,28]. The preparation of *E. bicyclis* powder involved a 24 h soak in tap water to eliminate foreign substances and salts, subsequent washing, and dehydration with hot air at 50 °C for 12 h. The dried *E. bicyclis* underwent powder formation through grinding, filtration, extraction with 80% MeOH, methanol volatilization, and freeze-drying in an ice maker at −80 °C, resulting in the production of the EB-Met extract. In the second phase, an equivalent amount of 100% n-hexane was added and stirred at 300 rpm at room temperature to separate the n-hexane layer from the upper layer. In the third step, an equal amount of 100% ethyl acetate was added to the removed water layer, followed by stirring. The supernatant was collected, concentrated under pressure (20–25 °C), and subsequently lyophilized at −80 °C. This process was repeated twice to yield the EB-EtOAc extract. The extraction yield was 10% from 1 kg of *E. bicyclis* powder during the fractionation process with 80% MeOH. Subsequently, 10 g of EB-EtOAc was obtained by fractionation with EtOAc, culminating in a final extraction yield of 1%.

2.2. LC-MS Analysis

LC-MS analysis was conducted using an LC system (6530 Infinity, Agilent Technologies, Santa Clara, CA, USA) coupled with a hybrid quadrupole time-of-flight mass spectrometer. A 10 μL injection of a 10 mg/mL sample solution was directly introduced onto an Atlantis T3 column (2.1 × 100 mm, 1.8 μm column) (Waters Inc., Milford, MA, USA) employing a gradient acetonitrile–water solvent system at room temperature. The mobile phase comprised acetonitrile–water in a gradient mode: acetonitrile (0.1% formic acid)–water (0.1% formic acid) (0–2 min: 95:5 *v*/*v*, 2–12 min: ~70:30 *v*/*v*, and 12–27 min: ~95:5) [29]. The flow rate was maintained at 0.3 mL/min, and the UV absorbance was detected at 280 nm.

2.3. Fermentation with Lactic Acid Bacteria

Lactobacillus acidophilus (LA), *Lactobacillus brevis* (LB), *Lactobacillus casei* (LC), and *Lactobacillus plantarum* subsp. plantarum (LP; Orla-Jensen 1919) were purchased from the Korean Collection for Type Cultures (Taejon, Republic of Korea). All lactic acid bacteria strains were cultured in an incubator at 37 °C for 24 h in MRS Broth medium. The absorbance was monitored until the mid-exponential stage of 0.6, which was determined by measuring the optical density (OD) at 600 nm. The extract EB-EtOAc (TA; 100 μg/mL), obtained through ethyl acetate extraction, was then mixed with four types of lactic acid species, LA, LB, LC, and LP. The mixtures were fermented for 24 h and subsequently filtered to collect the metabolites. After confirming that LC demonstrated the highest efficiency, the extract EB-EtOAc (TA) was fermented with LC for 24 h, followed by filtration. The resulting product was identified as TA + LC extract.

2.4. Cell Culture and In Vitro Anti-Inflammatory Assessment

For in vitro anti-inflammatory evaluation, lipopolysaccharide (LPS; *Escherichia coli* O55:B5; Sigma-Aldrich, St. Louis, MO, USA) at a concentration of 50 ng/mL was used to induce a cellular inflammation model, and a total of two experiments were performed for sample screening. The dosing concentration was determined by conducting a toxicity assay with RAW264.7 cells, selecting a concentration that showed a survival rate of more than 80%. RAW264.7 cells (1×10^5 cells/well) were seeded into 96-well plates and allowed to adhere for 24 h. The next day, LPS was administered to the cells with EB-Met extract and EB-EtoAc extract at 25 and 50 μg/mL concentrations (n = 8), and the supernatants were collected after 4 h and 20 h, respectively. A supernatant was used to evaluate the expression levels of TNF-α and IL-6. After selecting the extract, we performed the above

fermentation experiment to obtain the post-fermentation extract. The TA and fermentation extracts of four types of lactic acid bacteria (25 and 50 μg/mL) were evaluated for in vitro anti-inflammatory properties using the same experimental procedure (n = 8).

2.5. Animal Experimental Design

Male BALB/c mice (6–7 weeks old) were purchased from OrientBio (Seongnam, Gyeonggi-do, Republic of Korea) and housed in a facility with a 12 h light/dark cycle and temperature control. Following a one-week acclimatization period, the mice were randomly divided into five groups: control; dextran sulfate sodium (DSS) control; DTA (DSS + TA); DLC (DSS + LC); and DTL (DSS + TA + LC) (n = 8 per group). In the DTA and DTL groups, extract EB-EtOAc (TA) was administered by oral gavage for 4 weeks at a daily dose of 50 mg/kg body weight at 100 μL [30]. Conversely, the control, DSS, and DLC groups received 100 μL of PBS. To induce UC, a 3% DSS solution in drinking water was continuously administered for 7 days, starting on day 17. The DSS was replaced with water on day 24. In the colitis mice model, acute colitis was induced by administering 2–5% DSS for 3–7 days [31,32]. Studies investigating the effectiveness of bioactive compounds in suppressing UC typically administer 3% DSS in free drinking water for 7 days to induce UC symptoms in C57BL/6 mice [33,34]. Therefore, this concentration of DSS was utilized in our study. Throughout the DSS administration period, the body weight and Disease Activity Index (DAI) were assessed. Commencing on day 21, LC was orally administered via gavage feeding for 7 days (3rd week: 21–27 days; 1×10^8 CFU/100 μL). On day 30, all mice were euthanized, and serum, spleen, colon, and stool samples were collected for further analysis [35]. The animal protocol was approved by the Institutional Animal Care and Use Committee (IACUC: PNU2023-0420) of Pusan National University.

2.6. Histopathological Analysis

Distal colon samples from the mice were embedded in paraffin, stained with hematoxylin and eosin (H&E), and examined using a Nikon Eclipse Ts2R (Tokyo, Japan). Stained sections were independently evaluated by three trained investigators for the infiltration of inflammatory cells and the loss of crypts, and the scores were averaged. Histological scores were assessed based on the following criteria: epithelial hyperplasia and inflammatory cell infiltration: 0–3 scale (0 (normal) to 3 (pseudopolyps)); ulceration and mucosal crypt loss: 0–3 scale (0 (normal) to 3 (ulcerations)) [36].

2.7. ELISA Analysis

To determine the concentration of TNF-α and IL-6 released from RAW264.7 cells, commercial ELISA kits were employed (Catalog No. DY410, DY406; Assay Range: 31.2–2000 pg/mL; R&D Systems, Minneapolis, MN, USA). In brief, following in vitro treatment, 100 μL of cell media was collected at 4 h (for TNF-α) and 20 h (for IL-6) and centrifuged at 1200 rpm for 5 min. The supernatant was then added to 96-well plates with a capture antibody that had been placed the day before. Subsequent measurements were conducted in accordance with the manufacturer's instructions. For in vivo experiments, colon tissues were homogenized in buffer and centrifuged at 12,000 rpm for 10 min, and the supernatant was collected. The levels of inflammatory mediators, including IL-6, IFN-γ, and TNF-α, were assessed using ELISA, adhering to the manufacturer's guidelines (R&D Systems, Minneapolis, MN, USA). The quantification of colon cytokine levels was normalized to ng cytokine/mg colon protein (ng/mg). Serum samples were used to measure lipocalin-2 levels (R&D Systems).

2.8. Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR) and qPCR Analysis

Total RNA extraction from colon tissues was carried out using Trizol (Thermo Fisher, Waltham, MA, USA) and the RNeasy Mini Kit (Qiagen, Hilden, Germany). Herein, 1 μg of RNA underwent reverse transcription into cDNA utilizing the iScript cDNA Synthesis

Kit (Bio-Rad, Hercules, CA, USA). Gene amplification was detected through the CFX Connect Real-Time PCR Detection System (Bio-Rad). Primer design details can be found in Supplementary Table S1 (Macrogen, Seoul, Republic of Korea). The expression of target genes was normalized to β-actin expression. For gut microbiota composition analysis, total microbial DNA was extracted from feces using the Power Fecal Pro DNA Kit (Qiagen). Subsequently, 2 μg of DNA from bacteria was utilized in qPCR, with amplification conducted using primers (Macrogen). The analysis of gut microbial composition was performed through qPCR using the CFX Connect Real-Time PCR Detection System (Bio-Rad). The relative abundance of bacterial groups in each fecal sample was calculated as a percentage of total bacteria (F341/R518). Primer sequences are detailed in Supplementary Table S2.

2.9. Statistical Analysis

All data were analyzed using Prism 8 (GraphPad Software; La Jolla, CA, USA), and the results are presented as mean ± SD. The *p*-values were calculated using a one-way ANOVA (Dunnett's test), and the abundance of specific bacteria was determined using the non-parametric Kruskal–Wallis test. Statistical significance was set at $p < 0.05$.

3. Results

3.1. LC-MS Analysis of EB Extracts

The LC-MS analysis revealed distinct profiles for different extracts. In the EB-Met extract, we identified the presence of 7-phloroechol, phlorofucofuroecol A, and Dieckol (Figure 1A). Similarly, the EB-EtOAc extract contained 7-Phloroeckol, Phlorofucofuroeckol A, and Dieckol, with significantly elevated quantities by 334%, 250%, and 140%, respectively, compared to the EB-Met extract, indicating a richer phlorotannin fraction (Figure 1B). Additionally, the detection of phloroglucinol was confirmed in the EB-EtOAc extract. Upon fermentation, the EB-EtOAc extract exhibited an even more pronounced increase in phloroglucinol content along with the presence of 7-phloroechol and phlorofucofuroecol A (Figure 1C). This observation suggests a synergistic effect, with the fermented EB-EtOAc extract displaying the highest augmentation in phlorotannin levels compared to both EB-Met and non-fermented EB-EtOAc extracts. Notably, EB-EtOAc stood out for its diverse range of phlorotannins. Overall, our findings confirm the extraction of a phlorotannin-rich fraction, with the fermented EB-EtOAc extract demonstrating the most substantial increase in phloroglucinol content (Table 1).

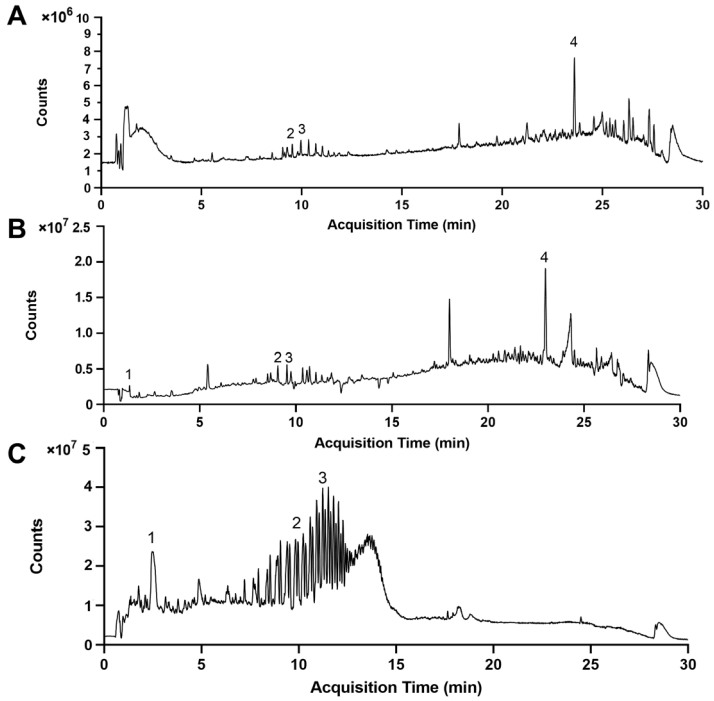

Figure 1. Representative LC-MS analysis results displayed in chromatogram. (**A**) *E. bicyclis* 80% methanol extract (EB-Met); (**B**) *E. bicyclis* ethyl acetate extract (EB-EtOAc); (**C**) fermented EB-EtOAc extract with *Lactobacillus casei* for 24 h. Peak 1: Phloroglucinol; Peak 2: 7-Phloroeckol; Peak 3: Phlorofucofuroeckol A; Peak 4: Dieckol.

Table 1. Chemical characterization of *E. bicyclis* extracts by LC-MS.

Peak	Compound Name	Molecular Formula	$[M + H]^+$	Fragment Ion (m/z)	EB-Met		EB-EtOAc		Fermented EB-EtOAC	
					Time (min)	Content *	Time (min)	Content *	Time (min)	Content *
1	Phloroglucinol	$C_6H_6O_3$	127.0389	127.0401, 127.0393, 128.0392	-	-	2.177	3.605	2.47	237.385
2	7-Phloroeckol	$C_{24}H_{16}O_{12}$	497.0703	498.0741, 499.0761, 499.0765	9.051	3.005	9.058	12.980	9.059	54.654
3	Phlorofucofuroeckol A	$C_{30}H_{18}O_{14}$	603.0763	604.0787, 605.0809	9.754	5.216	10.355	18.240	9.835	92.306
4	Dieckol	$C_{36}H_{22}O_{18}$	743.42	743.0865, 744.0898, 745.0923	22.98	37.275	22.985	89.707	-	-

* μg Phloroglucinol/mg extract.

3.2. Anti-Inflammatory Effects of Phlorotannins Isolated from E. bicyclis and Fermented Phlorotannins with Lactic Acid Bacteria Species

To investigate the isolation conditions and anti-inflammatory effects of high-purity phlorotannins from *E. bicyclis*, an experiment was conducted using LPS-treated RAW264.7 cells. The in vitro experiments tested the fractionation of phlorotannins from *E. bicyclis*, expressed as EB-Met or EB-EtOAc, based on the extraction conditions. In comparison to the PC group, TNF-α exhibited reductions of 21.3% and 35.5% in EB-Met (25 and 50 μg/mL, respectively), and 33.7% and 52.0% in EB-EtOAc (25 and 50 μg/mL, respectively). Notably, the reduction in inflammation was significantly higher in EB-EtOAc at 50 μg/mL (Figure 2A). IL-6 also showed reductions of 28.8% and 37.2% in EB-Met (25 and 50 μg/mL, respectively), and 36.7% and 40.9% in EB-EtOAc (25 and 50 μg/mL, respectively; Figure 2B). EB-EtOAc, representing a phlorotannin-rich fraction, consistently demonstrated superior anti-inflammatory effects compared with the EB-Met extract at all concentrations, exhibiting significant effects even at low concentrations.

Utilizing a fractionation process to obtain high-purity phlorotannins, we identified EB-EtOAc, distinguished by its remarkable anti-inflammatory effects, and abbreviated it as TA. To explore potential synergistic effects with lactic acid bacteria, TA and the four

selected strains were fermented for a 24 h period. Subsequently, the filtered extracts displayed a significant reduction in inflammation compared to that in the PC group. Compared to the PC group, the TNF-α levels exhibited reductions of 28.3% in TA, 24.3% in TA + LA, 52.1% in TA + LB, 50.7% in TA + LC, and 28.6% in TA + LP at a concentration of 50 μg/mL. Specifically, the levels of TNF-α in the TA + LB and TA + LC groups were significantly lower than those in the TA group (Figure 2C). Similarly, compared to the PC group, the IL-6 levels decreased by 29.3% in TA, 28.7% in TA + LA, 44.9% in TA + LB, 56.4% in TA + LC, and 39.0% in TA + LP at a concentration of 50 μg/mL. The level of IL-6 in the TA + LC groups was significantly lower than those in the TA group (Figure 2D). After comparison, it was found that the TA + LC combination exhibited the most effective anti-inflammatory effect. It was therefore considered that the LC strain showed the most effective synergy with TA, and the following experiments were conducted accordingly.

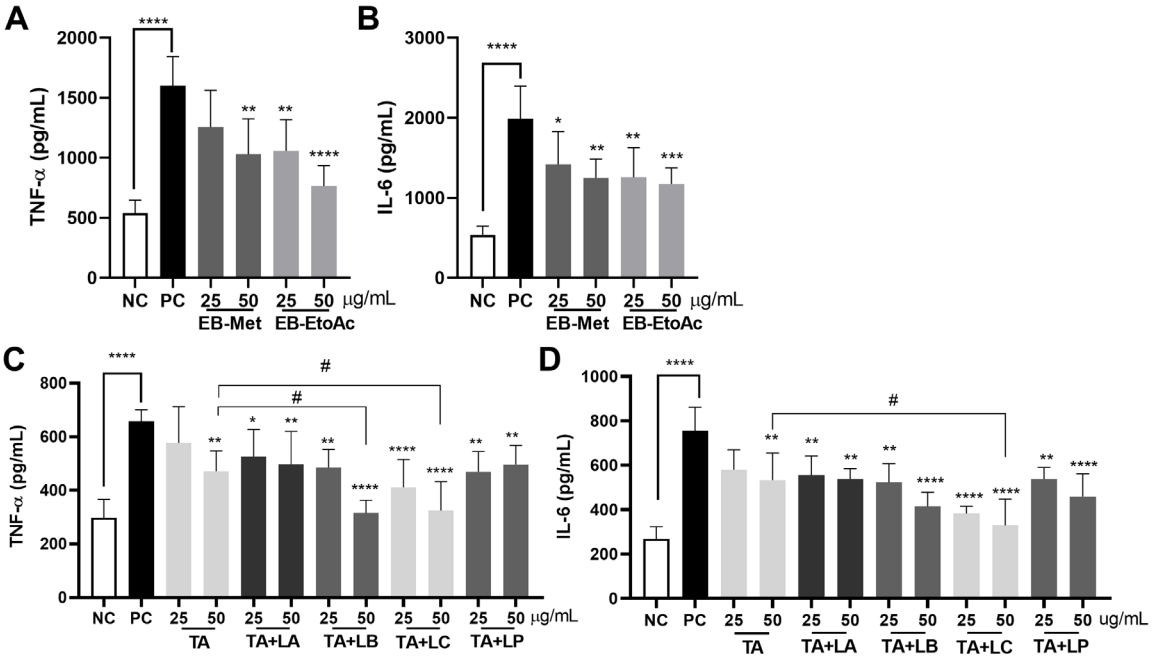

Figure 2. Anti-inflammatory effect of *E. bicyclis* extracts in LPS-treated RAW264.7 cells. (**A**) TNF-α and (**B**) IL-6 levels following treatment with differently isolated extracts from *E. bicyclis* (25–50 μg/mL). (**C**) TNF-α and (**D**) IL-6 levels after treating extracts fermented for 24 h with various lactic acid bacteria species. RAW264.7 cells were stimulated with LPS (50 ng/mL) and treated with the extracts (25–50 μg/mL) for 4–12 h. All data are expressed as means ± SD (n = 8/group). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ by one-way ANOVA followed by Dunnett's test (compared to the PC group). # $p < 0.05$ by Student t-test compared to the TA at the concentration of 50 μg/mL. NC: negative control; PC: positive control; EB-Met: *E. bicyclis* 80% methanol extract; EB-EtoAc: *E. bicyclis* ethyl acetate extract; TA: *E. bicyclis* ethyl acetate extract; LA: *Lactobacillus acidophilus*; LB: *Lactobacillus brevis*; LC: *Lactobacillus casei*; LP: *Lactobacillus plantarum.*

3.3. Effects of TA and LC on Body Weight, DAI Index, Colon Length, Spleen Weight, and Histological Score in DSS-Treated Mice

Following the in vitro experiments, we investigated the combined effects of the phlorotannin-rich fractions of *E. bicyclis* and LC in DSS-treated mice. A 3% DSS solution was administered for 7 days, starting on day 17. Rapid weight loss was observed 3 days after DSS administration. During DSS administration, the DSS group exhibited a weight loss rate of 22.7% on day 7 compared with that on day 0. In contrast, the control group, which did not receive DSS, showed a weight gain of 3.54%. The DTA, DLC, and DTL groups showed 8.64%, 11.3%, and 7.1% weight losses, respectively, which were significantly higher than those in the DSS group (Figure 3A,B). The DAI index was evaluated on a scale

of 0–3 for diarrhea and bloody stool and 0–3 for anal bleeding. Throughout the DSS administration period, the disease index improved in all extract treatment groups compared to the DSS group, with the lowest score observed in the DTL group (Figure 3C). Colon length, which is indicative of colon inflammation, was significantly increased in both the DTA and DTL groups compared to the DSS group (Figure 3D,E). Additionally, spleen enlargement was notably reduced by 31.3% in the DTL group relative to the DSS group, indicating recovery from the symptoms of spleen enlargement based on spleen weight, a major immune-related tissue (Figure 3F). Following the assignment of histological scores, the DTA and DTL groups exhibited significantly lower scores, indicating a reduced severity of intestinal tissue disease compared to the DSS group (Figure 3G,H).

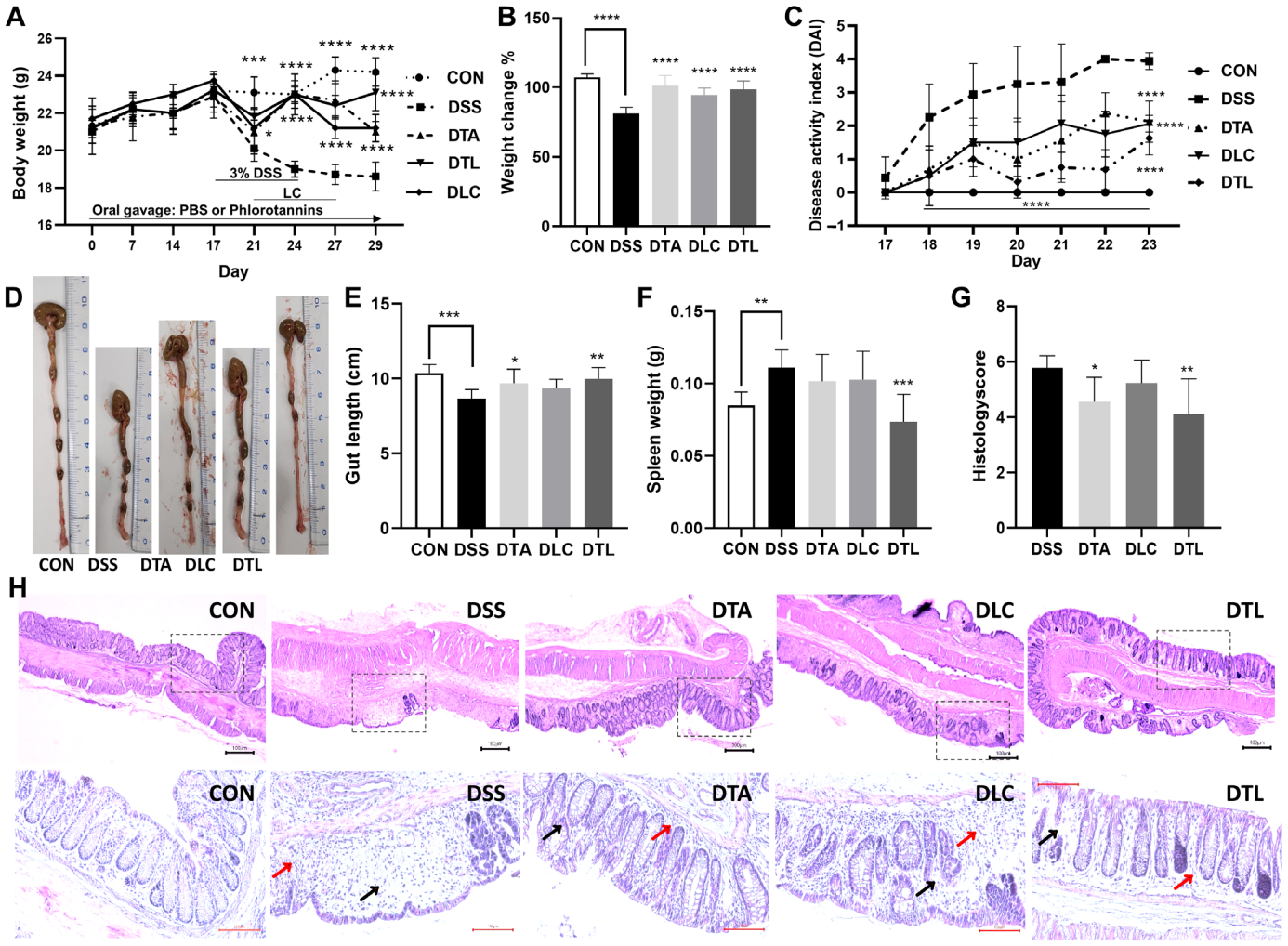

Figure 3. Effects of phlorotannins (TAs) isolated from *E. bicyclis* and *Lactobacillus casei* (LC) in DSS-treated mice. (**A**) Body weight changes. (**B**) Histogram showing proportion of weight change. (**C**) DAI index. (**D**) Colon length of each group of mice on the day of sacrifice. (**E**) Quantification chart for colon length. (**F**) Spleen weight. (**G**) Chart quantifying histological scoring. (**H**) Representative images of colon tissues. Black arrows indicate crypt loss, while red arrows indicate inflammatory cell infiltrates. Mice were fed experimental extracts (50 mg/kg bw/day) or PBS via oral gavage for 30 days. Subsequently, from day 17 to day 24, 3% dextran sulfate sodium (DSS) was provided in the drinking water, and *Lactobacillus casei* (1×10^8 cells/100 μL) was administered from day 21 to day 28. All data are expressed as mean ± SD ($n = 8$/group). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. One-way ANOVA followed by Dunnett's test. DSS: 3% DSS in drinking water for 7 days; DTA: DSS-Phlorotannin; DLC: DSS-*Lactobacillus casei*; DTL: DSS-Phlorotannin-*Lactobacillus casei*.

3.4. Effects of TA and LC on Inflammation Markers in DSS-Treated Mice

Serum lipocalin-2 serves as a reliable marker of inflammation. In comparison to DSS-treated mice, lipocalin-2 levels exhibited a decrease of 21.2% in DTA, 17.80% in DLC, and 55.7% in DTL, with the DTL group demonstrating the most substantial reduction (Figure 4A). Markers of UC, encompassing inflammation-related cytokines such as IL-6, TNF-α, and IFN-γ, representative of intestinal epithelial cells, were evaluated in colon tissue. The level of IFN-γ demonstrated a decrease of 44.2% in DTA, 39.3% in DLC, and 47.1% in DTL compared to the DSS group (Figure 4B). Additionally, in comparison to the DSS group, the level of TNF-α, a representative factor related to inflammation in UC, decreased by 20.7% in DTA, 62.9% in DLC, and 59.2% in DTL, with the DLC group exhibiting the most significant decrease (Figure 4C). However, no significant decrease in IL-6 levels was observed (Figure 4D).

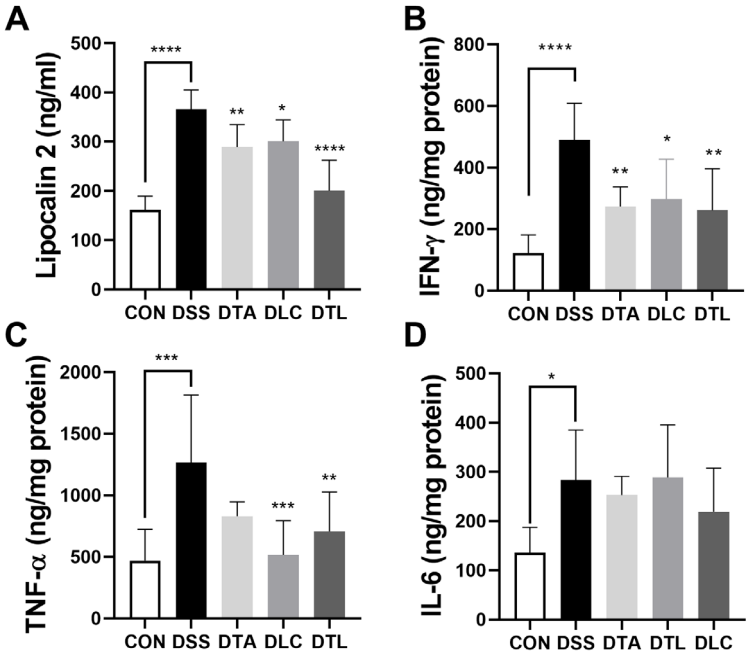

Figure 4. Effects of TA and LC on inflammatory cytokine levels in DSS-treated mice. (**A**) Lipocalin-2 in serum and (**B**) IFN-γ, (**C**) TNF-α, and (**D**) IL-6 levels in colon tissue of mice were measured by ELISA assay. Mice were fed experimental extracts (50 mg/kg bw/day) or PBS via oral gavage for 30 days. Subsequently, from day 17 to day 24, 3% dextran sulfate sodium (DSS) was provided in the drinking water, and *Lactobacillus casei* (1×10^8 cells/100 μL) was administered from day 21 to day 28. All data are expressed as mean ± SD (n = 8/group). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. One-way ANOVA followed by Dunnett's test. DSS: 3% DSS in drinking water for 7 days; DTA: DSS-Phlorotannin; DLC: DSS-*Lactobacillus casei*; DTL: DSS-Phlorotannin-*Lactobacillus casei*.

3.5. Effects of TA and LC on mRNA Expressions of Inflammatory Markers and Tight Junctions in DSS-Treated Mice

To determine the mechanisms underlying the combined effects of TA and LC, we evaluated the genetic factors implicated in the development of inflammation. RNA was extracted from the mouse colon tissue to assess the mRNA expression of key inflammatory markers and tight junction genes. The measured inflammatory genetic factors included Nfkb, Cox2, and iNos mRNA, which are representative inflammatory markers. Compared with the DSS group, Nfkb exhibited a significant reduction in inflammation in all extract treatment groups, with the DTL group demonstrating the most substantial reduction (Figure 5A). Cox-2 mRNA showed the most significant reduction, notably by 37.9%, in the DTL group compared to the DSS group (Figure 5B). The expression of iNOS demonstrated a decrease of 43.1% in DTA, 35.2% in DLC, and 70.5% in DTL compared to

the DSS group (Figure 5C). Moreover, tight junction genes (Mu2, Zo-1, Occludin, and Claudin1), crucial for protecting the epithelial barrier in inflammatory bowel disease, were assessed. The Muc2 gene increased by 29.3% in DTA and 64.2% in DTL, showing a significant improvement in tight junctions, specifically in the DTL group (Figure 5D). In comparison to the DSS group, all sample treatment groups exhibited an enhanced protective effect. Notably, the DTL group demonstrated a superior protective effect compared to the DTA and DLC groups, showing the highest significance in maintaining epithelial barrier integrity (Figure 5E–G).

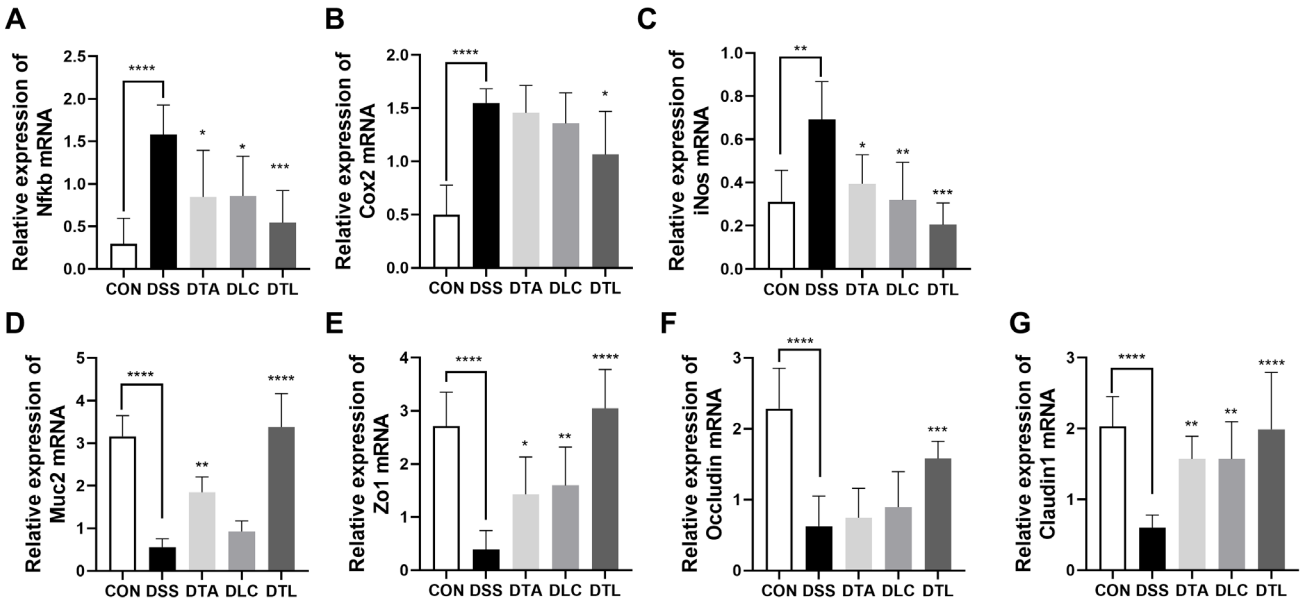

Figure 5. Effects of TA and LC on mRNA levels of pro-inflammatory factors and tight junctions in DSS-treated mice. (**A**) Nfkb, (**B**) Cox-2, (**C**) Inos, (**D**) Muc2, (**E**) Zo-1, (**F**) Occludin, and (**G**) Claudin1 mRNA expressions in mice colon tissue. β-actin was used as the reference gene. Mice were fed experimental extracts (50 mg/kg bw/day) or PBS via oral gavage for 30 days. Subsequently, from day 17 to day 24, 3% dextran sulfate sodium (DSS) was provided in the drinking water, and *Lactobacillus casei* (1×10^8 cells/100 μL) was administered from day 21 to day 28. All data are expressed as mean ± SD (n = 8/group). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. One-way ANOVA followed by Dunnett's test. DSS: 3% DSS in drinking water for 7 days; DTA: DSS-Phlorotannin; DLC: DSS-*Lactobacillus casei*; DTL: DSS-Phlorotannin-*Lactobacillus casei.*

3.6. Effects of TA and LC on the AhR Pathway in DSS-Treated Mice

To determine the mechanism underlying the combined effect of TA and LC in the gut, we examined the gut immune pathway with a focus on AhR. AHR and NRF2 interact with and regulate the expression of antioxidant genes containing DNA response elements. The potential antioxidant mechanism is triggered by the activation of AHR and NRF2, acting on the enzyme NQO1 [37]. The AhR gene exhibited a significant increase of 226% in DTA and 281% in DTL, indicating a notable enhancement following phlorotannin administration (Figure 6A). The expression of the antioxidant genes Nrf2 and Nqo1 was also significantly increased in all experimental groups (Figure 6B,C). The expression of IL-22, a crucial anti-inflammatory gene essential for the protection of colonic epithelial tissue and intestinal immunity, was significantly elevated in the DLC (93%), DTA (71%), and DTL (121%) groups compared to that in the DSS group (Figure 6D). DTA and DTL also inhibited the expression of Soc3, an inhibitor of cytokine signaling (Figure 6E). The expression of STAT3 in the DTL group was significantly increased by 79.2% compared to that in the DSS group (Figure 6F).

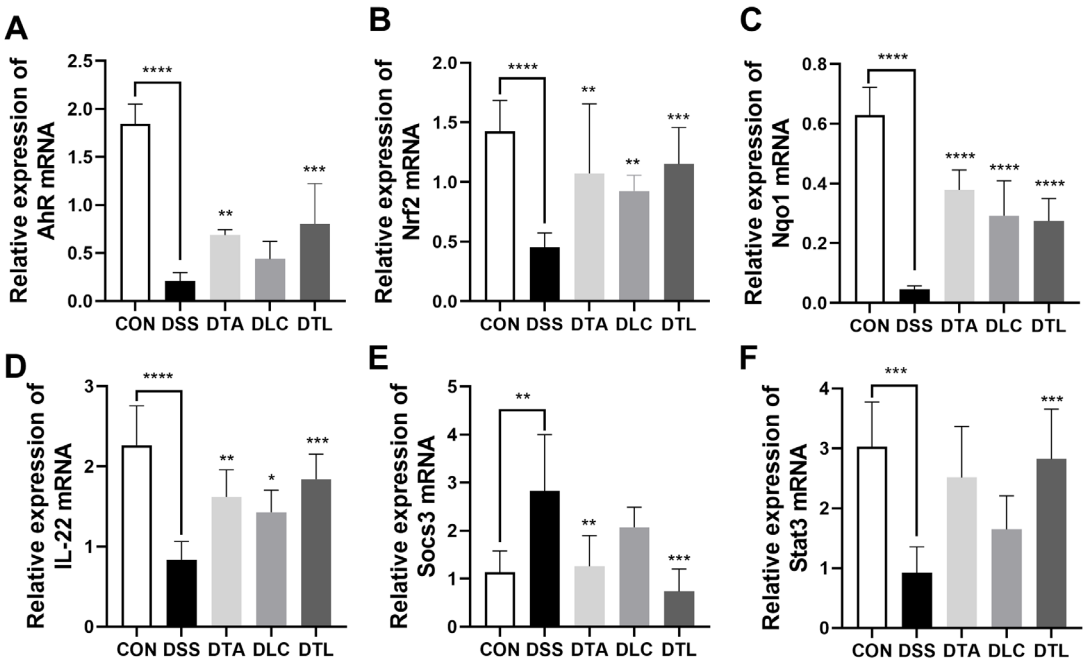

Figure 6. Effects of phlorotannins (TAs) isolated from *E. bicyclis* and *Lactobacillus casei* (LC) on mRNA expressions involved in the AhR pathway in DSS-treated mice. (**A**) AhR, (**B**) Nrf2, (**C**) Nqo1, (**D**) Il-22, (**E**) Socs3, and (**F**) Stat3 mRNA in colon tissue. β-actin was used as the reference gene. Mice were fed experimental extracts (50 mg/kg bw/day) or PBS via oral gavage for 30 days. Subsequently, from day 17 to day 24, 3% dextran sulfate sodium (DSS) was provided in the drinking water, and *Lactobacillus casei* (1×10^8 cells/100 μL) was administered from day 21 to day 28. All data are expressed as mean ± SD ($n = 8$/group). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. One-way ANOVA followed by Dunnett's test. DSS: 3% DSS in drinking water for 7 days; DTA: DSS-Phlorotannin; DLC: DSS-*Lactobacillus casei*; DTL: DSS-Phlorotannin-*Lactobacillus casei.*

3.7. Effects of TA and LC on the Fecal Microbiota Composition in DSS-Treated Mice

To measure the composition of the gut microbiota and the presence of beneficial bacteria, cDNA was extracted from the stool, and qPCR was performed. The Firmicutes/Bacteroides ratio, a representative indicator of intestinal condition, was optimized in the DTL group to closely resemble that of the control group (Figure 7A). Beneficial intestinal bacteria, including *Faecalibacterium prausnitzii*, *Akkermansia muciniphila*, *Lactobacillus plantarum*, and *Lactobacillus casei*, were significantly increased in all groups compared to the DSS group (Figure 7B–F). The abundance of *Lactobacillus casei* was particularly high in the LC-treated group, confirming the effectiveness of bacterial inoculation via oral gavage (Figure 7F). Moreover, *Lactobacillus reuteri*, known for its excellent immune effects, was significantly increased in the DTA and DTL groups (Figure 7D). This highlights the potential immunomodulatory effects of phlorotannins from *E. bicyclis* and *Lactobacillus casei* on gut microbiota.

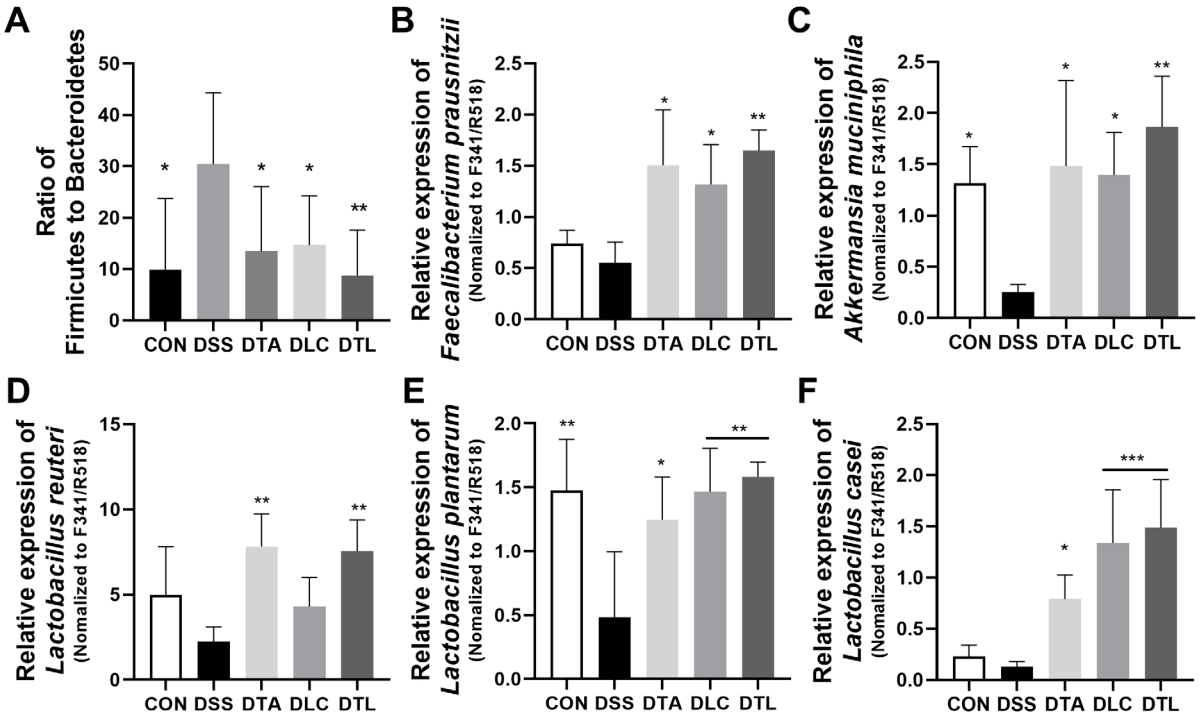

Figure 7. Effects of phlorotannins (TAs) isolated from *E. bicyclis* and *Lactobacillus casei* (LC) on the gut microbiota distribution in DSS-treated mice. The gut microbiota composition was quantitatively measured using the qPCR results for the selected bacterial group. (**A**) Firmicutes/Bacteroidetes ratio, (**B**) *Faecalibacterium prausnitzii,* (**C**) *Akkermansia muciniphila,* (**D**) *Lactobacillus reuteri*, (**E**) *Lactococcus plantarum*, (**F**) *Lactobacillus casei*. The relative abundance of bacterial groups in each fecal sample was expressed as a ratio of total bacteria (F341/R518). Mice were fed experimental extracts (50 mg/kg bw/day) or PBS via oral gavage for 30 days. Subsequently, from day 17 to day 24, 3% dextran sulfate sodium (DSS) was provided in the drinking water, and *Lactobacillus casei* (1×10^8 cells/100 μL) was administered from day 21 to day 28. All data are expressed as mean ± SD (n = 8/group). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ non-parametric Kruskal–Wallis test. DSS: 3% DSS in drinking water for 7 days; DTA: DSS-Phlorotannin; DLC: DSS-*Lactobacillus casei*; DTL: DSS-Phlorotannin-*Lactobacillus casei.*

4. Discussion

In this study, we used in vitro and in vivo colitis models to demonstrate that the combined administration of TA and LC exerted anti-inflammatory effects by modulating the AhR axis and influencing gut microbiota composition. Our study hypothesized that the synergistic effect of TA and LC fermentation metabolite uptake demonstrates that AhR regulates the gut microbiota through the basal axis, providing potent anti-inflammatory and protective effects on intestinal tight junctions, which was partially validated.

In this study, extracting TA from *E. bicyclis* using ethyl acetate after the initial methanol extraction increased the dieckol yield by 2.4 times, demonstrating the efficacy of this method for selectively isolating TA [38]. Fermenting the TA with LC resulted in the disappearance of the polymerized phlorotannin, dieckol, and the emergence of low-molecular-weight substances like phloroglucinol. Recognized for its excellent functional properties [39], phloroglucinol demonstrated anti-inflammatory effects in various experiments [40]. Since pure phloroglucinol might be absorbed in the small intestine and not effectively reach the colon when consumed directly or fermented externally before intake, we explored separately administering TA and LC. Another study indicated that high-molecular-weight phlorotannins potentially exert a stronger beneficial effect in the colon [41]. Notably, phlorotannin fermented with different Lactobacillus strains exhibited varying anti-inflammatory efficacy, suggesting variations in tannase enzyme production required

for tannin degradation depending on the types of Lactobacillus strains [42]. It was reported that synbiotic administration facilitates probiotic colonization of the gastrointestinal tract, while prebiotics optimize the proliferation of specific probiotics, enhancing their production of active metabolites in the intestine and providing superior intestinal protection [43]. In this in vitro study, TA fermented with LC demonstrated the most remarkable anti-inflammatory effect. In vivo PCR analysis confirmed an increase in *L. casei* abundance in the gut after consumption, indicating that phlorotannins, upon reaching the intestine, might undergo degradation, leading to increased metabolite production, including phloroglucinol, by augmented lactic bacteria. Phloroglucinol has been recently considered as one of the ligands that regulate AhR axis [25], a significant pathway involved in immune regulation within the intestines [44]. The synergistic effect of TA and LC may enhance the production of phloroglucinol from TA by LC when ingested simultaneously, thereby activating AhR to bolster immunity and repair tight junctions.

The DSS-induced colitis model, which is commonly used to induce acute and chronic UC, was used in this study because of its simplicity and similarity to human UC [31]. The DSS-induced control group showed characteristics of colitis such as neutrophil infiltration, barrier damage, disruption of tight junctions, inflammation, and dysbiosis of the gut microbiome. However, mice treated with the combination of TA and LC showed significant alleviation of the disease and improved treatment effects. Phlorotannins isolated from *E. Stolonifera* have previously demonstrated anti-inflammatory effects by inhibiting IL-1β, IL-6, and TNF-α, along with a reduction in NF-κB expression [45,46]. In the current study, the synergistic combination of TA and LC reduced inflammation, particularly in parameters such as spleen enlargement and lipocalin 2, Cox2, Occludin, and STAT3 mRNA expression, which are involved in inflammatory modulation and tight junction regulation.

The aryl hydrocarbon receptor (AhR) is a nuclear receptor that is pivotal in xenobiotic detoxification, immune regulation, and inflammation control. AhR activation results in the induction of cytochrome P450 1A1 (CYP1A1) and induces anti-inflammatory responses [47]. Currently, the AhR-Nrf2 and AhR-IL-22-STAT3 pathways have been highlighted for their potential to rescue intestinal barrier defects, regulate tight junctions, and suppress gastrointestinal inflammation, particularly in inflammatory bowel disease [44]. The Nrf2 signaling pathway, known for its role in cellular defense against oxidative stress, exhibits anti-inflammatory effects by blocking pro-inflammatory cytokines and the NF-κB pathway [48,49]. Additionally, the AhR/IL-22/STAT3 signaling pathway plays a vital role in gastrointestinal epithelial barrier function and the protection of intestinal stem cells [50,51]. Key transcription factors, including STAT3 and suppressor of cytokine signaling (SOCS), have been implicated in the transmission of inflammatory cytokine signals [52,53]. It has been shown that tight junctions and regulating Treg cell differentiation through the AhR/IL-22 pathway protects the colon from inflammatory damage in a mouse model of colitis, maintaining immune homeostasis [25]. Targeting these pathways is a promising strategy for the prevention and treatment of IBD. The metabolites produced by intestinal bacteria have also been recognized as integral components of AhR activation. Among these, the bacterial metabolites of tryptophan have been extensively studied, showcasing various physiological activities [54]. Additionally, polyphenols, another type of prebiotic ingredient, have garnered considerable attention as potential AhR ligands [25]. Quercetin induces a dose-dependent increase in the expression of tight junction (TJ) proteins ZO-1 and Claudin1 via the activation of AhR and CYP1A1 [25,55]. Baicalein has been shown to improve UC by enhancing the intestinal epithelial barrier through the AhR/IL-6 pathway in ILC3 [56,57]. Metabolites of ellagitannins demonstrated efficacy in mitigating colitis in preclinical models by preventing barrier dysfunction and showing their anti-inflammatory activity through the activation of the AhR-Nrf2-dependent pathway, upregulating epithelial tight junction proteins [58]. In this study, TA alone promoted AhR transcription and upregulated the expression of Nrf2, IL-22, and STAT3, while co-administration with LC resulted in a stronger induction of STAT3 and protection of tight

junctions. Research suggests that targeting these pathways, including AhR regulation, holds promise for the prevention and treatment of IBD. This effect may be attributed to the ability of LC to increase the production of metabolites, such as phloroglucinol, by metabolizing TA. However, further studies are required to fully understand the relevance of these pathways in intestinal diseases.

Intestinal dysbiosis and colitis are mutually correlated conditions. Studies have shown that polymerized polyphenols, including tannins, can effectively improve the intestinal flora environment and increase the abundance of bacteria like Bacteroides, Lactobacillus, Ruminococcaceae, and Akkermansia [59,60] that produce SCFAs. The phlorotannin selected in this study has been shown to have the ability to increase SCFAs [61]. SCFAs play crucial roles in regulating intestinal immune function, protecting the intestinal mucosal barrier, reducing inflammatory responses, maintaining intestinal environment stability, and treating various conditions such as IBD, hypertension, irritable bowel syndrome, and colon cancer. Furthermore, these polyphenols are suggested as AhR ligands, modulating the intestinal immune system through the AhR pathway [25]. Additionally, phlorotannin fermentation in an in vitro rumen model increased carbohydrate-mediated bacteria, including *Prevotellaceae_UCG-001*, *Anaerovorax*, *Ruminococcus*, *Ruminobacter*, *Fibrobacter*, *Lachnospiraceae_AC2044_group*, and *Clostridia_UCG-014* [62]. In this study, phlorotannin treatment increased the abundance of probiotics, including *F. prausnitzii*, *A. muciniphila*, *L. reuteri*, *L. casei*, and *L. plantarum*. *F. prausnitzii* is considered a key player in the gut, with significant effects on host health and immunity, as it produces anti-inflammatory molecules such as butyrate [63]. The role of *A. muciniphila* in treating intestinal inflammatory diseases, especially inflammatory bowel disease, has been widely recognized [64]. Lactobacillus species are representative probiotic strains in adjunctive IBD treatment. Multiple lactobacillus strains have been proven to reduce intestinal damage and enhance the intestinal immune barrier [65]. The Firmicutes/Bacteroidetes ratio is widely considered as crucial for maintaining normal intestinal homeostasis. Dysbiosis is often associated with an increase or decrease in this ratio. In the DSS group, the ratio significantly increased, whereas it significantly decreased after treatments. In summary, this study found that the co-administration of TA and LC significantly increased the content of various probiotics capable of treating colitis and adjusting the intestinal flora environment. This supports the notion that phlorotannin metabolites can maintain intestinal homeostasis and reduce inflammation by increasing the abundance of probiotic bacteria.

In this study, TA was administered to animals at a concentration of 50 mg/kg bw/day. A previous study administered dieckol at 5–15 mg/kg bw in DSS-induced colitis animal experiments, revealing efficacy against inflammation even at lower concentrations [11,66]. Additionally, another DSS mouse model using a phlorotannin-rich *Ecklonia cava* extract demonstrated anti-inflammatory effects ranging from 50 mg/kg bw up to 200 mg/kg bw [30]. In this study, the concentration of lactic acid bacteria was determined based on previous research, where a notable anti-inflammatory effect was observed after feeding *Lactobacillus plantarum* at a concentration of 1×10^9 CFU/mL for 10 days [67,68]. In an effort to establish optimal conditions, we administered approximately 1/10th of the amount used in the earlier experiment for a duration of approximately 7 days. Our selection of 50 mg/kg for this study is based on the preventive effects of phlorotannin on colitis. After converting the mouse doses to human-equivalent doses based on body surface area, to achieve similar effects, a 60 kg human would need to take 8 mg/kg bw, totaling 480 mg/day [69]. In studies involving other insomniacs and obese individuals, doses ranging from 100 mg to 500 mg per day were administered, with no reported toxic effects [70]. Therefore, it is unlikely that the 480 mg/day dose would lead to any adverse effects. Understanding the dose–response relationship is crucial for optimizing therapeutic applications. While in vitro results showed that different doses of TA and LC led to varying effects on inflammatory markers, our in vivo experiments were limited to testing only one concentration. Therefore, conducting additional dose–response studies is necessary to fully explore the potential for enhancing the anti-inflammatory effects of synbiotics. Future research avenues

may include investigating the synergistic effects between different doses of TA and LC, as well as exploring the impact of different dosing on treatment outcomes.

Our study's limitation lies in the low efficiency of extraction. Preliminary tests suggested higher efficiency when extracting *E. bicyclis* with 70% ethanol, contrasting with our primary objective to extract dieckol using the established method with 80% MeOH and 100% ethyl acetate [38,71]. Moreover, we solely monitored phlorotannin derivatives in the extract to evaluate the impact of probiotics on phlorotannin metabolism. While *E. bicyclis* is rich in phlorotannin, it also contains other bioactive compounds such as carotenoids, fucoxanthin, quercetin, and caffeic acid [72]. Further efforts are needed to investigate the amounts and effects of these other compounds, improve extraction efficiency, and enhance the purity of phlorotannin fractions. This study is limited by the absence of histological evidence demonstrating the rescue of tight junctions by TA and LC through modulation of the AhR pathway. Therefore, further investigations are needed to explore this aspect thoroughly. Another limitation of our study is the absence of a clear synergistic effect observed when administering the TA + LC combination, as compared to the TA alone group. Despite demonstrating excellent anti-inflammatory effects in in vitro experiments with fermented TA and LC, our initial hypothesis of a synergistic effect in animal experiments did not manifest fully. Only spleen size, Lipocalin 2, and TNF-α showed significant reductions when taken simultaneously. Moreover, tight junction-related genes like *Muc2*, *Zo1*, *Occludin*, etc., exhibited a significant increase following the combination of TA and LC compared to the tannin-only treatment, while other outcomes were similar to those observed with tannin intake alone. The difference in transitioning from in vitro to in vivo conditions can be attributed to two reasons: the methodology of compound delivery and the absorption and metabolism of the compounds in the digestive tract. Oral administration of Lactobacillus spp. and seaweed extracts may result in significant losses through the digestive tract due to variations in temperatures and pH values, potentially affecting the optimal delivery of bioactive compounds [73]. Additionally, differences in intestinal metabolism and the absorption capacity of metabolites may significantly contribute to the complexity of translating in vitro findings to in vivo settings [74].

In discussing the clinical relevance of the findings, it is crucial to consider the potential impact on the main symptoms of UC, which include inflammation, altered tight junction structure, and gut dysbiosis, and how these symptoms were mitigated by TA and LC administration. Phlorotannins have been demonstrated to possess anti-inflammatory properties, modulate the composition of the gut microbiota, and enhance intestinal barrier function [9,75]. Additionally, our results suggest that the combination of TA and LC may synergistically improve tight junctions. While individual studies have shown the efficacy of phlorotannins and LC in vivo and in clinical trials [70,76,77], the efficacy and safety of the combined administration in IBD has not been investigated. Based on our findings and the known properties of TA and LC, they could potentially serve as adjunctive therapies for human IBD. Further research is needed to assess the optimal administration and dosage regimens, as well as to ensure safety and efficacy in human subjects. In future research endeavors, our focus will be on refining the extraction method to enhance the extraction efficiency of phlorotannins. Following this optimization, we aim to conduct more mechanistic studies to thoroughly understand the AhR pathways through which TA and LC modulate intestinal health and inflammation. Additionally, we plan to investigate the dose–response relationship by evaluating the effects of different doses of TA and LC to assess their efficacy and safety. These efforts will be instrumental in facilitating the translation of our findings into clinical applications, ultimately advancing the potential therapeutic utility of TA and LC in managing inflammatory bowel diseases.

5. Conclusions

A combination of phlorotannins isolated from *E. bicyclis* and *Lactobacillus casei* was found to be highly effective in preventing ulcerative colitis. This was evidenced by the reduction in the expression of inflammatory cytokines and restoration of the ratio of Firmicutes and Bacteroidetes observed in experiments. The synergistic effects of the polyphenol components of the phlorotannins and *L. casei* was demonstrated to be superior to the individual effects of each component. Furthermore, cell experiments revealed that the polyphenol components of *E. bicyclis* and specific lactic acid bacterial species exhibit outstanding anti-inflammatory effects, emphasizing the importance of specific strains in conferring these benefits. In subsequent animal experiments, the combined administration of TA and LC not only protected the colon, but also increased the abundance of beneficial intestinal microorganisms, leading to a reduction in colon inflammation. The central role of the AhR axis in the combination of TA and LC suggests a positive effect on the gut immune axis, making it a potential candidate for an expanded symbiotic material in the management of IBD. The unique high-molecular-weight polyphenol component present in *E. bicyclis* interacts with specific strains of lactic acid bacteria, such as LC, and undergoes absorption and metabolism in the intestines. These findings highlight the potential of this combination as a functional food with protective properties against inflammatory diseases and other health conditions.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/app14072835/s1: Table S1: Sequences of primers used for quantitative RT-PCR primers; Table S2: Sequences of primers used for qPCR for bacterial profiling.

Author Contributions: Conceptualization and supervision, H.K.; methodology, Y.G.G., Q.W., and H.K.; investigation, Y.G.G., Q.W., and J.P.; data curation, Y.G.G. and Q.W.; writing—original draft preparation, Y.G.G.; writing—review and editing, Y.G.G., Q.W., H.-J.L., and H.K.; funding acquisition, H.-J.L. and H.K. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (No. 2021R1F1A1055822).

Institutional Review Board Statement: The animal protocol was approved by the Animal Ethics Committee of Pusan National University (PNU-2023-0420).

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available within the article.

Conflicts of Interest: There are no conflicts of interest to declare.

References

1. Seyedian, S.S.; Nokhostin, F.; Malamir, M.D. A review of the diagnosis, prevention, and treatment methods of inflammatory bowel disease. *J. Med. Life* **2019**, *12*, 113.
2. Khan, I.; Ullah, N.; Zha, L.; Bai, Y.; Khan, A.; Zhao, T.; Che, T.; Zhang, C. Alteration of gut microbiota in inflammatory bowel disease (IBD): Cause or consequence? IBD treatment targeting the gut microbiome. *Pathogens* **2019**, *8*, 126.
3. Tatiya-Aphiradee, N.; Chatuphonprasert, W.; Jarukamjorn, K. Immune response and inflammatory pathway of ulcerative colitis. *J. Basic Clin. Physiol. Pharmacol.* **2018**, *30*, 1–10.
4. K Ko, J.; K Auyeung, K. Inflammatory bowel disease: Etiology, pathogenesis and current therapy. *Curr. Pharm. Des.* **2014**, *20*, 1082–1096.
5. Khan, S.; Sebastian, S.A.; Parmar, M.P.; Ghadge, N.; Padda, I.; Keshta, A.S.; Minhaz, N.; Patel, A. Factors influencing the quality of life in inflammatory bowel disease: A comprehensive review. *Dis. Mon.* **2023**, *70*, 101672.
6. Ford, A.C.; Kane, S.V.; Khan, K.J.; Achkar, J.-P.; Talley, N.J.; Marshall, J.K.; Moayyedi, P. Efficacy of 5-aminosalicylates in Crohn's disease: Systematic review and meta-analysis. *Am. J. Gastroenterol.* **2011**, *106*, 617–629.
7. Chowdhury, M.T.H.; Bangoura, I.; Kang, J.-Y.; Cho, J.Y.; Joo, J.; Choi, Y.S.; Hwang, D.S.; Hong, Y.-K. Comparison of Ecklonia cava, Ecklonia stolonifera and Eisenia bicyclis for phlorotannin extraction. *J. Environ. Biol.* **2014**, *35*, 713.
8. Nakamura, T.; Nagayama, K.; Uchida, K.; Tanaka, R. Antioxidant activity of phlorotannins isolated from the brown alga Eisenia bicyclis. *Fish. Sci.* **1996**, *62*, 923–926.
9. Liao, M.; Wei, S.; Hu, X.; Liu, J.; Wang, J. Protective Effect and Mechanisms of Eckol on Chronic Ulcerative Colitis Induced by Dextran Sulfate Sodium in Mice. *Mar. Drugs* **2023**, *21*, 376.

10. Thomas, N.V.; Kim, S.-K. Potential pharmacological applications of polyphenolic derivatives from marine brown algae. *Environ. Toxicol. Pharmacol.* **2011**, *32*, 325–335.
11. Zhu, X.; Sun, Y.; Zhang, Y.; Su, X.; Luo, C.; Alarifi, S.; Yang, H. Dieckol alleviates dextran sulfate sodium-induced colitis via inhibition of inflammatory pathway and activation of Nrf2/HO-1 signaling pathway. *Environ. Toxicol.* **2021**, *36*, 782–788.
12. Cherry, P.; Yadav, S.; Strain, C.R.; Allsopp, P.J.; McSorley, E.M.; Ross, R.P.; Stanton, C. Prebiotics from seaweeds: An ocean of opportunity? *Mar. Drugs* **2019**, *17*, 327.
13. Shen, S.; Yang, W.; Li, L.; Zhu, Y.; Yang, Y.; Ni, H.; Jiang, Z.; Zheng, M. In vitro fermentation of seaweed polysaccharides and tea polyphenol blends by human intestinal flora and their effects on intestinal inflammation. *Food Funct.* **2023**, *14*, 1133–1147.
14. Yang, B.; Li, M.; Wang, S.; Ross, R.P.; Stanton, C.; Zhao, J.; Zhang, H.; Chen, W. Lactobacillus ruminis alleviates DSS-induced colitis by inflammatory cytokines and gut microbiota modulation. *Foods* **2021**, *10*, 1349.
15. Tabashsum, Z.; Peng, M.; Bernhardt, C.; Patel, P.; Carrion, M.; Biswas, D. Synbiotic-like effect of linoleic acid overproducing Lactobacillus casei with berry phenolic extracts against pathogenesis of enterohemorrhagic Escherichia coli. *Gut Pathog.* **2019**, *11*, 1–11.
16. Corona, G.; Ji, Y.; Anegboonlap, P.; Hotchkiss, S.; Gill, C.; Yaqoob, P.; Spencer, J.P.; Rowland, I. Gastrointestinal modifications and bioavailability of brown seaweed phlorotannins and effects on inflammatory markers. *Br. J. Nutr.* **2016**, *115*, 1240–1253.
17. Mahmoud, A.M.; Bin-Jumah, M.; Abukhalil, M.H. Antiinflammatory natural products from marine algae. In *Inflammation and Natural Products*; Elsevier: Amsterdam, The Netherlands, 2021; pp. 175–203.
18. Li, H.; Christman, L.M.; Li, R.; Gu, L. Synergic interactions between polyphenols and gut microbiota in mitigating inflammatory bowel diseases. *Food Funct.* **2020**, *11*, 4878–4891.
19. Fei, Y.; Zhang, S.; Han, S.; Qiu, B.; Lu, Y.; Huang, W.; Li, F.; Chen, D.; Berglund, B.; Xiao, H. The role of dihydroresveratrol in enhancing the synergistic effect of ligilactobacillus salivarius Li01 and resveratrol in ameliorating colitis in mice. *Research* **2022**, *2022*, 9863845.
20. Wang, H.; Fan, C.; Zhao, Z.; Zhai, Z.; Hao, Y. Anti-inflammatory effect of Bifidobacterium animalis subsp. lactis A6 on DSS-induced colitis in mice. *J. Appl. Microbiol.* **2022**, *133*, 2063–2073.
21. Qiu, J.; Heller, J.J.; Guo, X.; Zong-ming, E.C.; Fish, K.; Fu, Y.-X.; Zhou, L. The aryl hydrocarbon receptor regulates gut immunity through modulation of innate lymphoid cells. *Immunity* **2012**, *36*, 92–104.
22. Yu, M.; Wang, Q.; Ma, Y.; Li, L.; Yu, K.; Zhang, Z.; Chen, G.; Li, X.; Xiao, W.; Xu, P. Aryl hydrocarbon receptor activation modulates intestinal epithelial barrier function by maintaining tight junction integrity. *Int. J. Biol. Sci.* **2018**, *14*, 69.
23. Neavin, D.R.; Liu, D.; Ray, B.; Weinshilboum, R.M. The role of the aryl hydrocarbon receptor (AHR) in immune and inflammatory diseases. *Int. J. Mol. Sci.* **2018**, *19*, 3851.
24. Wang, X.; Xie, X.; Li, Y.; Xie, X.; Huang, S.; Pan, S.; Zou, Y.; Pan, Z.; Wang, Q.; Chen, J. Quercetin ameliorates ulcerative colitis by activating aryl hydrocarbon receptor to improve intestinal barrier integrity. *Phytother. Res.* **2023**, *38*, 253–264.
25. Pinto, C.G.; Ávila-Gálvez, M.Á.; Lian, Y.; Moura-Alves, P.; Dos Santos, C.N. Targeting the aryl hydrocarbon receptor by gut phenolic metabolites: A strategy towards gut inflammation. *Redox Biol.* **2023**, *61*, 102622.
26. Fukumoto, S.; Toshimitsu, T.; Matsuoka, S.; Maruyama, A.; Oh-oka, K.; Takamura, T.; Nakamura, Y.; Ishimaru, K.; Fujii-Kuriyama, Y.; Ikegami, S. Identification of a probiotic bacteria-derived activator of the aryl hydrocarbon receptor that inhibits colitis. *Immunol. Cell Biol.* **2014**, *92*, 460–465.
27. Abdelhamid, A.; Lajili, S.; Elkaibi, M.A.; Ben Salem, Y.; Abdelhamid, A.; Muller, C.D.; Majdoub, H.; Kraiem, J.; Bouraoui, A. Optimized extraction, preliminary characterization and evaluation of the in vitro anticancer activity of phlorotannin-rich fraction from the brown seaweed, Cystoseira sedoides. *J. Aquat. Food Prod. Technol.* **2019**, *28*, 892–909.
28. Leyton, A.; Pezoa-Conte, R.; Barriga, A.; Buschmann, A.; Mäki-Arvela, P.; Mikkola, J.-P.; Lienqueo, M. Identification and efficient extraction method of phlorotannins from the brown seaweed Macrocystis pyrifera using an orthogonal experimental design. *Algal Res.* **2016**, *16*, 201–208.
29. Steevensz, A.J.; MacKinnon, S.L.; Hankinson, R.; Craft, C.; Connan, S.; Stengel, D.B.; Melanson, J.E. Profiling phlorotannins in brown macroalgae by liquid chromatography–high resolution mass spectrometry. *Phytochem. Anal.* **2012**, *23*, 547–553.
30. Kim, Y.-M.; Kim, H.-Y.; Jang, J.-T.; Hong, S. Preventive Effect of Ecklonia cava Extract on DSS-Induced Colitis by Elevating Intestinal Barrier Function and Improving Pathogenic Inflammation. *Molecules* **2023**, *28*, 8099.
31. Chassaing, B.; Aitken, J.D.; Malleshappa, M.; Vijay-Kumar, M. Dextran sulfate sodium (DSS)-induced colitis in mice. *Curr. Protoc. Immunol.* **2014**, *104*, 15.25.11–15.25.14.
32. Perše, M.; Cerar, A. Dextran sodium sulphate colitis mouse model: Traps and tricks. *Biomed Res. Int.* **2012**, *2012*, 718617.
33. Chen, Y.; Zhang, P.; Chen, W.; Chen, G. Ferroptosis mediated DSS-induced ulcerative colitis associated with Nrf2/HO-1 signaling pathway. *Immunol. Lett.* **2020**, *225*, 9–15.
34. Peng, L.; Gao, X.; Nie, L.; Xie, J.; Dai, T.; Shi, C.; Tao, L.; Wang, Y.; Tian, Y.; Sheng, J. Astragalin attenuates dextran sulfate sodium (DSS)-induced acute experimental colitis by alleviating gut microbiota dysbiosis and inhibiting NF-κB activation in mice. *Front. Immunol.* **2020**, *11*, 2058.
35. Im, Y.; Wang, Q.; Park, J.; Lee, H.; Kim, H. Sargassum horneri Extract Ameliorates DSS-Induced Colitis through Modulation of mTOR Axis and Intestinal Microbiota. *Appl. Sci.* **2023**, *13*, 1742.
36. Erben, U.; Loddenkemper, C.; Doerfel, K.; Spieckermann, S.; Haller, D.; Heimesaat, M.M.; Zeitz, M.; Siegmund, B.; Kühl, A.A. A guide to histomorphological evaluation of intestinal inflammation in mouse models. *Int. J. Clin. Exp. Pathol.* **2014**, *7*, 4557.
37. Dietrich, C. Antioxidant functions of the aryl hydrocarbon receptor. *Stem Cells Int.* **2016**, *2016*, 7943495.

38. Catarino, M.D.; Silva, A.M.; Mateus, N.; Cardoso, S.M. Optimization of phlorotannins extraction from Fucus vesiculosus and evaluation of their potential to prevent metabolic disorders. *Mar. Drugs* **2019**, *17*, 162.
39. Kumar, L.R.; Paul, P.T.; Anas, K.; Tejpal, C.; Chatterjee, N.; Anupama, T.; Mathew, S.; Ravishankar, C. Phlorotannins–bioactivity and extraction perspectives. *J. Appl. Phycol.* **2022**, *34*, 2173–2185.
40. Kim, M.-M.; Kim, S.-K. Effect of phloroglucinol on oxidative stress and inflammation. *Food Chem. Toxicol.* **2010**, *48*, 2925–2933.
41. Corona, G.; Coman, M.M.; Guo, Y.; Hotchkiss, S.; Gill, C.; Yaqoob, P.; Spencer, J.P.; Rowland, I. Effect of simulated gastrointestinal digestion and fermentation on polyphenolic content and bioactivity of brown seaweed phlorotannin-rich extracts. *Mol. Nutr. Food Res.* **2017**, *61*, 1700223.
42. Ren, B.; Wu, M.; Wang, Q.; Peng, X.; Wen, H.; McKinstry, W.J.; Chen, Q. Crystal structure of tannase from Lactobacillus plantarum. *J. Mol. Biol.* **2013**, *425*, 2737–2751.
43. Markowiak, P.; Śliżewska, K. Effects of probiotics, prebiotics, and synbiotics on human health. *Nutrients* **2017**, *9*, 1021.
44. Hou, J.-J.; Ma, A.-H.; Qin, Y.-H. Activation of the aryl hydrocarbon receptor in inflammatory bowel disease: Insights from gut microbiota. *Front. Cell. Infect. Microbiol.* **2023**, *13*, 1279172.
45. Khan, F.; Jeong, G.-J.; Khan, M.S.A.; Tabassum, N.; Kim, Y.-M. Seaweed-Derived phlorotannins: A review of multiple biological roles and action mechanisms. *Mar. Drugs* **2022**, *20*, 384.
46. Kim, B.; Choi, C.-G.; Kim, J.-I.; Kim, H.-R. Anti-Inflammatory Effect of Hexane Fraction from Eisenia bicyclis on Lipopolysaccharides-Treated RAW 264.7 Cells. *Fish Aquat. Sci.* **2021**, *54*, 152–161.
47. Manzella, C.; Singhal, M.; Alrefai, W.A.; Saksena, S.; Dudeja, P.K.; Gill, R.K. Serotonin is an endogenous regulator of intestinal CYP1A1 via AhR. *Sci. Rep.* **2018**, *8*, 6103.
48. Yuan, Z.; Yang, L.; Zhang, X.; Ji, P.; Hua, Y.; Wei, Y. Huang-lian-jie-du decoction ameliorates acute ulcerative colitis in mice via regulating NF-κB and Nrf2 signaling pathways and enhancing intestinal barrier function. *Front. Pharmacol.* **2019**, *10*, 1354.
49. Liu, D.; Huo, X.; Gao, L.; Zhang, J.; Ni, H.; Cao, L. NF-κB and Nrf2 pathways contribute to the protective effect of Licochalcone A on dextran sulphate sodium-induced ulcerative colitis in mice. *Biomed. Pharmacother.* **2018**, *102*, 922–929.
50. Pickert, G.; Neufert, C.; Leppkes, M.; Zheng, Y.; Wittkopf, N.; Warntjen, M.; Lehr, H.-A.; Hirth, S.; Weigmann, B.; Wirtz, S. STAT3 links IL-22 signaling in intestinal epithelial cells to mucosal wound healing. *J. Exp. Med.* **2009**, *206*, 1465–1472.
51. Sugimoto, K.; Ogawa, A.; Mizoguchi, E.; Shimomura, Y.; Andoh, A.; Bhan, A.K.; Blumberg, R.S.; Xavier, R.J.; Mizoguchi, A. IL-22 ameliorates intestinal inflammation in a mouse model of ulcerative colitis. *J. Clin. Investig.* **2008**, *118*, 534–544.
52. Li, Y.; de Haar, C.; Peppelenbosch, M.P.; van der Woude, C.J. SOCS3 in immune regulation of inflammatory bowel disease and inflammatory bowel disease-related cancer. *Cytokine Growth Factor Rev.* **2012**, *23*, 127–138.
53. Wang, J.; Wang, P.; Tian, H.; Tian, F.; Zhang, Y.; Zhang, L.; Gao, X.; Wang, X. Aryl hydrocarbon receptor/IL-22/Stat3 signaling pathway is involved in the modulation of intestinal mucosa antimicrobial molecules by commensal microbiota in mice. *Innate Immun.* **2018**, *24*, 297–306.
54. Salminen, A. Activation of aryl hydrocarbon receptor (AhR) in Alzheimer's disease: Role of tryptophan metabolites generated by gut host-microbiota. *J. Mol. Med.* **2023**, *101*, 201–222.
55. Oleaga, C.; García, M.; Solé, A.; Ciudad, C.J.; Izquierdo-Pulido, M.; Noé, V. CYP1A1 is overexpressed upon incubation of breast cancer cells with a polyphenolic cocoa extract. *Eur. J. Nutr.* **2012**, *51*, 465–476.
56. Li, Y.-y.; Wang, X.-j.; Su, Y.-l.; Wang, Q.; Huang, S.-w.; Pan, Z.-f.; Chen, Y.-p.; Liang, J.-j.; Zhang, M.-l.; Xie, X.-q. Baicalein ameliorates ulcerative colitis by improving intestinal epithelial barrier via AhR/IL-22 pathway in ILC3s. *Acta Pharmacol. Sin.* **2022**, *43*, 1495–1507.
57. Wang, X.; Huang, S.; Zhang, M.; Su, Y.; Pan, Z.; Liang, J.; Xie, X.; Wang, Q.; Chen, J.; Zhou, L. Gegen Qinlian decoction activates AhR/IL-22 to repair intestinal barrier by modulating gut microbiota-related tryptophan metabolism in ulcerative colitis mice. *J. Ethnopharmacol.* **2023**, *302*, 115919.
58. Singh, R.; Chandrashekharappa, S.; Bodduluri, S.R.; Baby, B.V.; Hegde, B.; Kotla, N.G.; Hiwale, A.A.; Saiyed, T.; Patel, P.; Vijay-Kumar, M. Enhancement of the gut barrier integrity by a microbial metabolite through the Nrf2 pathway. *Nat. Commun.* **2019**, *10*, 89.
59. Wang, X.; Qi, Y.; Zheng, H. Dietary polyphenol, gut microbiota, and health benefits. *Antioxidants* **2022**, *11*, 1212.
60. Kong, C.; Gao, R.; Yan, X.; Huang, L.; Qin, H. Probiotics improve gut microbiota dysbiosis in obese mice fed a high-fat or high-sucrose diet. *Nutrition* **2019**, *60*, 175–184.
61. Charoensiddhi, S.; Conlon, M.A.; Vuaran, M.S.; Franco, C.M.; Zhang, W. Polysaccharide and phlorotannin-enriched extracts of the brown seaweed Ecklonia radiata influence human gut microbiota and fermentation in vitro. *J. Appl. Phycol.* **2017**, *29*, 2407–2416.
62. Huang, Q.; Chen, Y.; Wang, X.; Wei, Y.; Pan, M.; Zhao, G. Effects of Phlorotannins from Sargassum on In Vitro Rumen Fermentation, Microbiota and Fatty Acid Profile. *Animals* **2023**, *13*, 2854.
63. Leylabadlo, H.E.; Ghotaslou, R.; Feizabadi, M.M.; Farajnia, S.; Moaddab, S.Y.; Ganbarov, K.; Khodadadi, E.; Tanomand, A.; Sheykhsaran, E.; Yousefi, B. The critical role of Faecalibacterium prausnitzii in human health: An overview. *Microb. Pathog.* **2020**, *149*, 104344.
64. Zhang, T.; Ji, X.; Lu, G.; Zhang, F. The potential of Akkermansia muciniphila in inflammatory bowel disease. *Appl. Microbiol. Biotechnol.* **2021**, *105*, 5785–5794.
65. Li, C.; Peng, K.; Xiao, S.; Long, Y.; Yu, Q. The role of Lactobacillus in inflammatory bowel disease: From actualities to prospects. *Cell Death Discov.* **2023**, *9*, 361.

66. Rajan, D.K.; Mohan, K.; Zhang, S.; Ganesan, A.R. Dieckol: A brown algal phlorotannin with biological potential. *Biomed. Pharmacother.* **2021**, *142*, 111988.
67. Xia, Y.; Chen, Y.; Wang, G.; Yang, Y.; Song, X.; Xiong, Z.; Zhang, H.; Lai, P.; Wang, S.; Ai, L. Lactobacillus plantarum AR113 alleviates DSS-induced colitis by regulating the TLR4/MyD88/NF-κB pathway and gut microbiota composition. *J. Funct. Foods* **2020**, *67*, 103854.
68. Al-Sadi, R.; Nighot, P.; Nighot, M.; Haque, M.; Rawat, M.; Ma, T.Y. Lactobacillus acidophilus induces a strain-specific and toll-like receptor 2–dependent enhancement of intestinal epithelial tight junction barrier and protection against intestinal inflammation. *Am. J. Pathol.* **2021**, *191*, 872–884.
69. Nair, A.B.; Jacob, S. A simple practice guide for dose conversion between animals and human. *J. Basic Clin. Physiol. Pharmacol.* **2016**, *7*, 27.
70. Negara, B.F.S.P.; Sohn, J.H.; Kim, J.-S.; Choi, J.-S. Effects of phlorotannins on organisms: Focus on the safety, toxicity, and availability of phlorotannins. *Foods* **2021**, *10*, 452.
71. Jung, H.A.; Jin, S.E.; Ahn, B.R.; Lee, C.M.; Choi, J.S. Anti-inflammatory activity of edible brown alga Eisenia bicyclis and its constituents fucosterol and phlorotannins in LPS-stimulated RAW264. 7 macrophages. *Food Chem. Toxicol.* **2013**, *59*, 199–206.
72. Raja, R.; Hemaiswarya, S.; Arunkumar, K.; Mathiyazhagan, N.; Kandasamy, S.; Arun, A.; Ramasamy, P. Efficacy of Eisenia bicyclis phlorotannins in the treatment of diabetes and reducing inflammation. *Food Biosci.* **2023**, *52*, 102381.
73. Yang, L.; Chu, J.S.; Fix, J.A. Colon-specific drug delivery: New approaches and in vitro/in vivo evaluation. *Int. J. Pharm.* **2002**, *235*, 1–15.
74. Xu, Y.; Shrestha, N.; Préat, V.; Beloqui, A. An overview of in vitro, ex vivo and in vivo models for studying the transport of drugs across intestinal barriers. *Adv. Drug Deliv. Rev.* **2021**, *175*, 113795.
75. Zhu, Y.; Guo, J.; Hu, X.; Liu, J.; Li, S.; Wang, J. Eckol protects against acute experimental colitis in mice: Possible involvement of Reg3g. *J. Funct. Foods* **2020**, *73*, 104088.
76. Um, M.Y.; Kim, J.Y.; Han, J.K.; Kim, J.; Yang, H.; Yoon, M.; Kim, J.; Kang, S.W.; Cho, S. Phlorotannin supplement decreases wake after sleep onset in adults with self-reported sleep disturbance: A randomized, controlled, double-blind clinical and polysomnographic study. *Phytother. Res.* **2018**, *32*, 698–704.
77. D'Incà, R.; Barollo, M.; Scarpa, M.; Grillo, A.R.; Brun, P.; Vettorato, M.G.; Castagliuolo, I.; Sturniolo, G.C. Rectal administration of Lactobacillus casei DG modifies flora composition and Toll-like receptor expression in colonic mucosa of patients with mild ulcerative colitis. *Dig. Dis. Sci.* **2011**, *56*, 1178–1187.