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INHIBITION OF COLONIC INFLAMMATION BY EDIBLE SEAWEEDS

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Inhibition of colonic inflammation by edible seaweeds

A Dissertation Presented

by

Lingxiao Yi

Submitted to the Graduate School of the
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Food Science

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Dedication

To my dear father, mother, family members and friends

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to my advisor, Prof. Hang Xiao, for providing me with an opportunity to fulfill my dream in his group. With his kind guidance, his motivation, his continuous support and insight on science, I was trained and prepared to be a mature scientific researcher. I cannot complete this work without his guidance and help. I treasure the time and experience in his research group. Also, I would like to sincerely thank my committee members, Dr. Eric Decker, Dr. John Gibbons and Dr. Zhenhua Liu, for their kindly guidance, valuable time, recommendations that improve the quality of this dissertation.

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ABSTRACT

INHIBITION OF COLONIC INFLAMMATION BY EDIBLE SEAWEEDS

MAY 2023

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The pathogenesis of inflammatory bowel diseases (IBD) has not been completely understood. Nevertheless, the current hypotheses were that the intestinal homeostasis among microbiota, immune cells and intestinal epithelial cells are disrupted by multiple factors, including genetic predisposition, immune, environmental and microbiome, resulting in a dysregulated inflammation. Recent evidence suggests dietary patterns play vital roles in the disease progression of IBD, and amelioration of IBD-associated dysbiosis of gut microbiota by dietary components may help prevent and/or treat IBD. Edible seaweed, a type of marine crop rich in various macronutrients and micronutrients, confer health benefits to the host against chronic diseases, namely cancer, inflammatory disease, cardiovascular disease and obesity. Large amounts of studies have reported that components in seaweed possess protective effects against IBD and prebiotic effects on IBD-associated gut microbiota. The present study focuses on the beneficial roles of seaweed bioactive compounds in IBD and IBD-associated gut microbiota dysbiosis and aims to fill the knowledge

gap in the dietary prevention of IBD by seaweeds and seaweed products.

Polyphenols from edible seaweed display various health benefits which have not been adequately studied. The current study aimed to characterize the composition of extractable polyphenol-rich components (EPCs) and non-extractable polyphenol-rich components (NEPCs) from three edible seaweeds (*Laminaria japonica*, *Ulva lactuca* and *Porphyra tenera*) and evaluate their anti-inflammatory and anti-colon cancer properties. Both EPCs and NEPCs from three edible seaweeds against lipopolysaccharides (LPS) stimulated nitric oxide in macrophages. Further mechanistic tests revealed that EPCs and NEPCs regulated the expression levels of proinflammatory enzymes, proinflammatory cytokines and antioxidant enzymes in macrophages. Furthermore, EPCs and NEPCs lowered the viability of colon cancer cells, while normal colon cells were not affected. Additionally, EPCs and NEPCs induced cellular apoptosis and led to G0/G1 cell cycle arrest in HCT116 cells. Overall, these results provide a rationale for future animal and human studies designed to examine the anti-inflammatory and chemo-preventive capacities of polyphenol-rich components from *L. japonica*, *U. lactuca* and *P. tenera*.

Laminaria japonica (LJ) and *Porphyra tenera* (PT) are two major edible seaweeds with various health benefits. The current study designed to evaluate the anti-colitis properties of whole LJ and PT in dextran-sulfate-sodium (DSS)-treated mice. Supplementation of LJ and PT were found to against the loss of body weight, suppress the disease activity index (DAI), ameliorate the colonic histological injuries in colitis mice, which was linked to the reduction of the levels of pro-inflammatory cytokines and inflammatory-related proteins in the colon mucosa. Moreover, supplementation of edible seaweeds was found to alter gut microbiota diversity and composition in colitis mice. Specifically,

supplementation of LJ and PT elevated the proportion of potential beneficial bacteria, such as *Lactobacillus*, *Blautia* and *Roseburia*, as well as lowered the relative abundance of *Enterococcus*, *Escherichia-Shigella* and *Akkermansia* in colitis mice. Additionally, supplementation of LJ and PT reversed the dysregulation of short-chain fatty acids (SCFAs) and bile acids (BAs) in colitis mice. Overall, these results illustrate efficacy and mechanism behind the anti-colitis effects of LJ and PT, which provide a scientific basis for the prevention of inflammatory agents.

TABLE OF CONTENTS

	PAGE
ACKNOWLEDGEMENTS	v
ABSTRACT.....	vi
LIST OF TABLES.....	xiii
LIST OF FIGURES	xiv
CHAPTER	
1. INTRODUCTION.....	1
1.1. Interaction among IBD, gut microbiota and dietary pattern	4
1.2. Bioactive compounds from seaweeds against IBD.....	8
1.3. Seaweed modulates IBD-associated gut microbiome.....	16
1.4. Major bioactive compounds versus whole seaweed against IBD	25
2. INHIBITORY EFFECTS OF POLYPHENOLS-RICH COMPONENTS FROM THREE EDIBLE SEaweeds ON INFLAMMATION AND COLON CANCER IN VITRO	28
2.1. Introduction.....	28
2.2. Materials and Methods.....	30
2.2.1 Materials	30
2.2.2 Preparation of polyphenols-rich components.....	30
2.2.3 Evaluation of total phenolics contents (PCs), flavonoids contents (FCs), tannins contents (TCs), carbohydrates contents (CCs), and proteins content (PRCs)	31

2.2.4 Evaluation of antioxidant properties of EPCs and NEPCs	33
2.2.5 Identification of phenolic compounds.....	34
2.2.6 Cytotoxicity and nitrite oxide assay of RAW 264.7 cells	34
2.2.7 Cell viability of normal colon cells and colon cancer cells	35
2.2.8 Flow cytometer analysis	35
2.2.9 qRT-PCR analysis	35
2.2.10 Immunoblotting.....	36
2.2.11 Statistically analysis.....	36
2.3. Results and Discussion	36
2.3.1 Chemical profiles of EPCs and NEPCs in <i>L. japonica</i> , <i>U. lactuca</i> and <i>P. tenera</i>	36
2.3.2 Antioxidant capacities of the EPCs and NEPCs in <i>L. japonica</i> , <i>U. lactuca</i> and <i>P. tenera</i>	42
2.3.3 EPCs and NEPCs reduced the NO production in activated macrophages	42
2.3.4 EPCs and NEPCs lowered the gene expression of proinflammatory cytokines	45
2.3.5 EPCs and NEPCs suppressed iNOS and COX-2 expression in activated macrophages	46
2.3.6 EPCs and NEPCs elevated the expression levels of antioxidant enzymes in activated macrophages	47
2.3.7 EPCs and NEPCs suppressed the viability of colon cancer cells.....	48
2.3.8 EPCs and NEPCs led cell cycle arrest and apoptosis	50
3. DIETARY INTAKE OF <i>LAMINARIA JAPONICA</i> ATTENUATED DEXTRAN SULFATE SODIUM-INDUCED ACUTE COLITIS IN MICE	53

3.1. Introduction.....	53
3.2. Materials and methods	54
3.2.1 Materials	54
3.2.2 Animals and experimental design	55
3.2.3 Assessment of disease activity index (DAI) and colitis severity	56
3.2.4 Assessment of inflammatory enzymes and cytokines in colon tissues	56
3.2.5 Quantification of SCFAs.....	56
3.2.6 Quantification of BAs	57
3.2.7 Analysis of fecal microbiota	57
3.2.8 Statistically analysis.....	58
3.3. Results and discussion	58
3.3.1 Supplementation of LJ inhibited the symptoms of colitis.....	58
3.3.2 Supplementation of LJ regulated the production of pro-inflammatory cytokines and inflammatory-related proteins.....	61
3.3.3 Supplementation of LJ ameliorated the production of BAs and SCFAs.....	63
3.3.4 Supplementation of LJ reversed the gut microbiota dysbiosis.....	67
3.3.5 Correlation analysis between the gut microbiota and colitis indices	74
4. ANTI-INFLAMMATORY PROPERTIES OF PORPHYRA TENERA IN MICE WITH DEXTRAN SODIUM SULFATE-INDUCED COLITIS	76
4.1. Introduction.....	76
4.2. Materials and Methods.....	77
4.2.1 Materials	77

4.2.2	Animals and experimental design	78
4.2.3	Disease activity index (DAI) and colonic histology analysis	79
4.2.4	Enzyme-linked immunosorbent and immunoblotting assay	79
4.2.5	Analysis of gut microbiota	79
4.2.6	Determination of short-chain fatty acids (SCFAs).....	80
4.2.7	Quantification of bile acids (BAs)	80
4.2.8	statistically analysis	81
4.3.	Results.....	81
4.3.1	Intake of PT relieve the symptoms of colitis	81
4.3.2	Intake of PT regulated the inflammatory-related protein and pro-inflammatory cytokines	83
4.3.3	Intake of PT shifted the gut microbiota diversity.....	85
4.3.4	Intake of PT reversed the gut microbiota structure	87
4.3.5	Intake of PT shifted the production of SCFAs and BAs	91
4.3.6	Correlation analysis between the gut microbiota and colitis indices	95
4.4	Discussion.....	96
5.	CONCLUSION AND FUTURE PERSPECTIVES	100
	BIBLIOGRAPHY	102

LIST OF TABLES

Table	Page
Table.1. Impact of seaweed extracts against IBD in in vivo studies.....	11
Table.2. Impact of bioactive compounds from seaweed on gut microbiota in in vitro studies	18
Table.3. Impact of bioactive compounds from seaweed on gut microbiota in in vivo studies	22
Table.4. Primers pairs used for cDNA amplification	35
Table.5. Phenolic compounds identified in the EPCs and NEPCs in three edible seaweeds.	41
Table.6. BAs and SCFAs content in different groups administrated by LJ.	65
Table.7. Relative abundance of gut microbiota with significant differences among different group	73
Table.8. Relative abundance of gut microbiota at with significant differences in each group	89
Table.9. SCFAs and BAs content in different groups treated by PT.....	93

LIST OF FIGURES

Figure	Page
Figure.1. The relationship between oral administration of seaweeds and gut microbiota homeostasis in the disease progression of IBD.....	3
Figure.2. Total phenolic contents (PCs) (A), flavonoid contents (FCs) (B), tannin contents (TCs) (C), carbohydrate content (CCs) (D), protein contents (PRCs) (E) in EPCs and NEPCs from three edible seaweeds. The levels of ORAC (F), DPPH (G) and ABTS (H) of the EPCs and NEPCs from three edible seaweeds.....	39
Figure.3. Inhibitory effects of EPCs from <i>L. japonica</i> (A), <i>U. lactuca</i> (B), <i>P. tenera</i> (C) on NO production in activated macrophages. Inhibitory effects of NEPCs from <i>L. japonica</i> (D), <i>U. lactuca</i> (E), <i>P. tenera</i> (F) on NO production in activated macrophages.....	44
Figure.4. Suppressive effects of EPCs and NEPCs from <i>L. japonica</i> (A), <i>U. lactuca</i> (B), <i>P. tenera</i> (C) on mRNA expression of TNF- α , IL-6, IL-1 in activated macrophages.....	45
Figure.5. Effects of EPCs and NEPCs from <i>L. japonica</i> (A), <i>U. lactuca</i> (B), <i>P. tenera</i> (C) on mRNA expression of iNOS, COX-2, HO-1 and NQO-1 in activated macrophages.....	47
Figure.6. Effects of EPCs and NEPCs from <i>L. japonica</i> (A), <i>U. lactuca</i> (B), <i>P. tenera</i> (C) on protein expression of iNOS, COX-2 and HO-1 in activated macrophages.....	48
Figure.7. Effects of the EPCs and NEPCs from <i>L. japonica</i> (A), <i>P. tenera</i> (B) and <i>U. lactuca</i> (C) on the growth of CCD18-Co cells for 72 hours; Suppressive effects of the EPCs and NEPCs from <i>L. japonica</i> (D), <i>P. tenera</i> (E) and <i>U. lactuca</i> (F) on HCT116 cells for 48 hours; Suppressive effects of the EPCs and NEPCs from <i>L. japonica</i> (D), <i>P. tenera</i> (E) and <i>U. lactuca</i> (F) on HCT116 cells for 72 hours.....	50
Figure.8. Quantification of cell cycle distribution posed to EPCs and NEPCs from <i>L. japonica</i> (A), <i>U. lactuca</i> (B), and <i>P. tenera</i> (C) and their representative DNA histograms of cell cycle treatment. Quantification of early and late apoptosis posed to EPCs and NEPCs from <i>L. japonica</i> (D), <i>U.</i>	

<i>lactuca</i> (E), <i>P. tenera</i> (F) and their representative Annexin V/PI co-stain dot plots of cell apoptosis treatment.	52
Figure.9. Supplementation of LJ improves the symptoms of DSS-induced acute colitis. (A) Experimental design; (B) the change of body weight (n = 10 per group); (C) DAI score (n = 10 per group); (D) spleen weights (n = 8 -10 per group); (E) colon length (n = 8 – 10 per group).....	60
Figure.10. LJ supplementation ameliorated colonic histology damage and regulated the boost of pro-inflammatory cytokines. (A) Representative image of H&E-stained colon (300 × magnification); (B) Histology score of colon damage (n = 6); (C) Quantification of inflammatory cytokines (IL-1 β , IL-2, IL-6, TNF- α , IFN- γ) in colon mucosa (n = 6). (D) Western blotting analysis of inflammatory-related proteins in the colon mucosa (n = 3).....	63
Figure.11. (A) Concentrations of primary BAs in mice feces (n = 5); (B) Concentrations of Secondary BAs in mice feces (n = 5); (C) Concentrations of SCFAs in mice cecal contents (n = 5).	67
Figure.12. LJ supplementation partially reversed gut microbiota dysbiosis in DSS-treated mice. (A) Modulatory effects of LJ on the α -diversity of gut microbiota, including number of OTUs, Shannon index, Simpson index and Chao 1 index (n = 5); (B) Modulatory effects of LJ on the beta diversity of gut microbiota in healthy mice, presented as principal coordinates analysis (PCoA) plot (n = 5). (C) Modulatory effects of LJ on the beta diversity of gut microbiota in colitis mice, presented as PCoA plot (n = 5). (D) taxonomic cladogram obtained from LEfSe analysis ($\alpha < 0.1$); (E) Distribution of histogram based on the LDA scores (LDA score > 4 and $\alpha < 0.1$).....	70
Figure.13. LJ supplementation partially reverses the composition of gut microbiota. (A) Relative abundance of gut microbiota at the Phylum level (n = 5); (B) Relative abundance of Bacteroidetes, Firmicutes, Proteobacteria and Actinobacteria (n = 5); (C) Relative abundance of <i>Lactobacillus</i> , <i>Blautia</i> , <i>Akkermansia</i> , <i>Escherichia-Shigella</i> and <i>Enterococcus</i> at genus levels.....	72
Figure.14. The correlation between the bacterial and cytokines or SCFAs or BAs.....	75

Figure.15. (A) The change of body weight during the entire experiments in different groups; (B) DAI scores in different groups; (C) Final body weight; (D) Spleen weight; (E) Colon length; (F) Representative image of H&E-stained colon (300 × magnification); (G) Histology scores of colonic damages.	82
Figure.16. (A) Quantification of the pro-inflammatory cytokines in colonic mucosa (n = 8); (B) Western blotting analysis of inflammatory-related protein in colon mucosa (n = 8).....	84
Figure.17. (A) PT alters the α -diversity of gut microbiota with various indices; (B) PT shifts the β -diversity of gut microbiota in healthy mice, visualized with PCoA plots; (C) PT shifts the β -diversity of the gut microbiota in colitis mice, presented as PCoA plots; (D) Taxonomic cladogram obtained from LEfSe analysis ($\alpha < 0.1$); (E) Taxa with LDA score > 4.	86
Figure.18. (A) The distribution of gut microbiota at the Phylum level in colitis mice (n = 5); (B) Relative abundance of Firmicutes and Bacteroidetes in colitis mice (n = 5); (C) Relative abundance of <i>Escherichia-Shigella</i> , <i>Enterococcus</i> , <i>Oscillibacter</i> , <i>Lactobacillus</i> , <i>Blautia</i> and <i>Roseburia</i> in colitis mice (n =5). (D) Relative abundance of <i>Enterobacteriaceae</i> , <i>Ruminococcaceae</i> , <i>Enterococcaceae</i> , <i>Lactobacillaceae</i> and <i>Lachnospiraceae</i> in colitis mice (n =5).	91
Figure.19. Concentrations of SCFAs (A), primary BAs (B) and secondary BAs (C) in different groups of mice.	95
Figure.20. Heatmap of the correlation between the bacterial and cytokines or SCFAs or BAs....	96

CHAPTER 1

INTRODUCTION

Inflammatory bowel diseases (IBD), consisting of ulcerative colitis (UC) and Crohn's disease (CD), is a chronic immune-mediated disorder in gastrointestinal tract [1]. IBD is a globally health issue with a higher prevalence in the Europe and North America [2, 3]. Meanwhile, the incidence of IBD is increasing rapidly in Asia, Africa and the Middle East [4]. UC normally only involved in the rectum, and the common presentation is the superficial ulcer caused by localized inflammatory reactions in the mucosa. CD commonly involved in the whole gastrointestinal tract, and the presentations include deep ulceration, fistula and stenosis [5]. Currently available medical treatments, especially for 5-aminosalicylate agents (5-ASA), antibiotics, corticosteroids, nonsteroidal anti-inflammatory drugs (NSAID), immunomodulators, are widely adopted to active IBD patients [6, 7]. Nevertheless, these drug therapy in IBD treatment is challenged by distinct side-effects and poor tolerance in patient lifetime [8].

Up to now, the pathogenesis of IBD is largely unclear. Nevertheless, the current hypotheses is that the homeostasis among microbiota, immune cells and intestinal epithelial cells are disrupted by multiple factors, including genetic, immune, environmental and microbiome, resulting in a dysregulated inflammation [9]. IBD appears partially caused by the imbalance of the immune response which is characterized by epithelial damage, immune cells infiltration and the expansion of inflammation. For instance, macrophages, dendritic cells, T cells and B cells play a vital role in regulating the inflammatory responses [10]. Meanwhile, gut microbiota is related to the pathogenesis of IBD. More than 200 IBD

associated genomic loci have been identified, most of them are known to be involved in regulating host response to gut microbiota [11, 12]. Additionally, environmental factors, involving smoking, antibiotics, diet and urbanization, elevate the susceptibility of IBD [13]. Among them, dietary pattern is an important factor in the disease progression of IBD. Accumulating studies indicated that dietary pattern plays a vital role in promoting the colon health by modifying gut microbiota structure and producing bioactive metabolites, thereby controlling the development of IBD [14, 15]. Increasing the consumption of fruits, vegetables, olive oil and amino acids have been associated with the lower risk of IBD [16], and positively affects gut microbiota via inhibiting the growth of pathogenic bacteria and/or promoting the growth of beneficial bacteria [17, 18].

Seaweeds, including approximately 25,000 species, can be divided into three taxonomic groups, as green seaweeds (Chlorophyceae), red seaweeds (Rhodophyceae) and brown seaweeds (Phaeophyceae), according to their colorant pigments [19]. Seaweed cultivation has increased rapidly, which does not require arable land, freshwater, or fertilizer. Thus, it becomes a unique and sustainable source for the food and nutraceutical industry, different from traditional edible terrestrial plants [20]. The application of seaweeds as foodstuff for human health and nutrition trace back to several hundred years ago in Asian countries [21]. Seaweeds are rich in bioactive components, including polysaccharides, proteins, minerals, polyphenols and polyunsaturated fatty acids, subjected to many studies for their anti-inflammatory, antioxidant, anticancer, immunomodulatory and prebiotic effects [22]. In addition, seaweeds and their extracts, especially for polysaccharides and polyphenols, can serve as a substrate for certain species of gut microbiota, producing various metabolites, including short-chain fatty acids (SCFAs) and gases, which can, in turn, affect the gut

microbiota composition [23] (Figure.1). Importantly, many bioactive compounds from seaweeds are found to pose protective effects on IBD in mouse studies in recent years [24-26]. Thus, edible seaweeds may be used as an alternative approach for regulating IBD-induced dysbiosis of gut microbiota and preventing IBD. Here we present the current information on the roles of bioactive compounds from seaweeds in IBD and IBD-associated gut microbiota. This is to fill the knowledge gap in the dietary prevention of IBD by seaweeds and seaweed products.

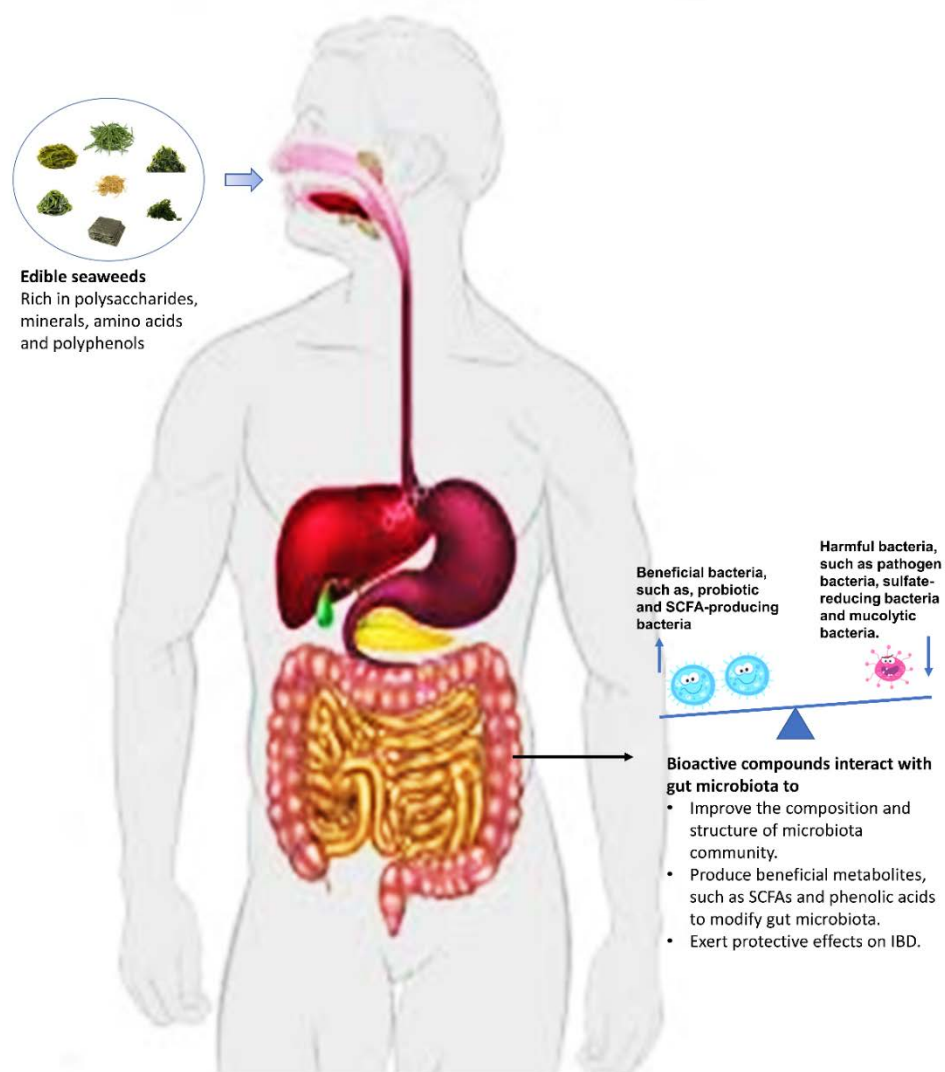


Figure.1. The relationship between oral administration of seaweeds and gut microbiota homeostasis in the disease progression of IBD. Seaweeds are rich in polysaccharides,

minerals, amino acids and polyphenols. As a result, bioactive compounds resistant to digestion in the small intestine reach the colon, interacting with gut microbiota. This interaction may lead to modulating gut microbiota composition and structure, regulating the production of beneficial metabolites, such as SCFAs and phenolic acids. Moreover, the complex interaction between seaweed and gut microbiota may play a vital role in the disease progression of IBD by changing gut permeability, barrier function, and the balance of the intestinal immune system.

1.1. Interaction among IBD, gut microbiota and dietary pattern

Diet is known to be an important factor in maintaining and promoting health for the host. Large amounts of studies have identified that food components, including carbohydrates, proteins, lipids and phytochemicals have a great influence on the growth of gut microbiota and have been shown to exert protective effects on the development of IBD [27, 28]. The current section provides an overview of the interaction among the IBD, gut microbiota and dietary pattern, which has been comprehensively studied during past decades. Edible terrestrial plants, including isolated bioactive compounds and whole foods, have been found to promote colon health in disease-free populations, involving enhancing the diversity and composition of gut microbiota, and have been shown to exert their protective effects on IBD in various experimental settings, including altering the IBD-associated dysbiosis of gut microbiota, and altered gut microbiota-derived metabolites [29-33]. Further studies established the role of gut microbiota in regulating anti-inflammatory effects of intake bioactive compounds from the edible terrestrial plants in colitis mice [34, 35].

The gut microbiota plays a vital role in maintaining human health. For example, gut microbiota supplies vitamin K and energy sources, generates metabolic products from dietary compounds and develops immune systems [36]. Gut microbiota is mainly comprised of bacteria, and most of them belong to the phyla of Firmicutes and Bacteroidetes,

where the phyla of Proteobacteria, Actinobacteria coexist in healthy individuals [37]. Recent studies have enhanced our insight into the interplay between IBD progression and gut microbiota by altering the epithelial barriers, immune response, and inflammation [38, 39]. In the host, a balance among different groups of bacteria is required to maintain health. The shift of balance of gut microbiota is known as dysbiosis, and gut microbiota dysbiosis is a typical hallmark of IBD. Gut microbiota dysbiosis disrupts the bacterial community, trigger the abnormal immune response, such as releasing inflammatory factors, then alter the gut microbiota-derived metabolites, such as SCFAs, bile acids and tryptophan, thereby resulting in IBD [40]. Many studies have elucidated that the diversity of gut microbiota and their metabolites in IBD patients were different, when compared with healthy individuals [41-43]. Overall, the increase of bacteria with pro-inflammatory properties and the decrease of bacteria with anti-inflammatory properties are observed in IBD patients [44].

The most consistent alterations are a decrease in Firmicutes and an increase Proteobacteria [44, 45]. Both an increase and a decrease in the abundance of Bacteroidetes have been observed in IBD patients [46]. The potential pathogens, including *Helicobacter*, adherent invasive *E.coli* and *Escherichia-Shigella* are highly associated with IBD [47, 48]. The presence of adherent invasive *E. coli* stimulates immune responses, and alters the intestine permeability and the diversity of gut microbiota, consequently leading to IBD [49]. Also, an increased abundance of mucolytic bacteria, including *Ruminococcus gnavas* and *Ruminococcus torques*, have been reported in IBD patients due to the alteration of permeability of intestine [50]. The production of microbiota metabolites is also linked to the disease progression of IBD. For instance, the concentrations of SCFAs were reduced in IBD patients, as a result of a decreased abundance of SCFA-producing bacteria, such as *F.*

prausnitzii and *Clostridium* clusters IV, XIVa, XVIII [51]. A reduced SCFAs production affects the growth of epithelial cells and the differentiation of regulatory T cells [52]. An elevated abundance of the members of sulfate-reducing bacteria, such as *Desulfovibrio*, was reported in the IBD patients. Accumulated production of hydrogen sulfate disrupts epithelium cells and induces chronic inflammation [53, 54]. Interestingly, an increased abundance of the probiotic, including *Bifidobacterium* and *Lactobacillus*, have been reported in IBD patients. The accumulation of probiotic have been shown to stimulate the production of anti-inflammatory factors that promote the recovery of the intestinal barrier, protect against harmful bacteria, and improve the immune response resulting in the reduction of intestinal inflammation and mitigation of the symptoms of IBD [55]. To date, several innovative alternative strategies to prevent or ameliorate IBD have been presented based on the increased understanding of the intestinal microbiota, including the application of fecal microbiota transplantation, and antibiotic, probiotic, and symbiotic approaches [56]. In general, all the above findings highlight the key role of gut microbiota in IBD regulation.

Recently, studies have indicated that diet is linked to IBD-associated dysbiosis of the gut microbiota [15]. Different dietary patterns have been found to shape their typical gut microbiota composition and structure, thus forming different health states of the body. For example, the Western diet, which contains higher amount of fats, is linked to an increased risk for IBD. Fat is known to alter gut microbiota composition, and high-fat diets reduce the abundance of Bacteroidetes and increase the abundance of Firmicutes and Proteobacteria [57]. Moreover, certain genera of bacteria, such as *Lactobacillus*, *Bifidobacterium*, and *Faecalibacterium* are generally reduced under a high-fat diet. These bacteria

suppress the inflammatory response in intestine via lowering the expression of pro-inflammatory cytokines, such as IL-6 and IL-1, and stimulating the production of anti-inflammatory cytokines, such as IL-10 and TGF- β [58, 59]. In contrast, a high intake of fruits and vegetables, rich in polysaccharides, polyphenols and other micronutrients, may inhibit the IBD via regulation of IBD-related gut microbiota. Administration of broccoli (10% w/w), blueberry (10% w/w), strawberry (5% w/w), and cranberry (1.5% w/w) in diets slightly reduced the fecal abundance of *C. perfringens* and *E. coli* and increased the fecal abundance of *Lactobacillus* and *Bifidobacterium*, as well as the production of SCFAs in colitis mice [30, 60, 61]. The major compounds in fruits and vegetables that modulate the composition of gut microbiota are microbiota-accessible carbohydrates (MAC) and polyphenols. MAC are not digested in the small intestine, reach the colon nearly intactly. Consequently, these compounds serve as a substrate for the gut microbiota, enhancing the diversity and composition of gut microbiota, and leading to the alteration of metabolites, such as SCFAs [62, 63]. Similarly, the bioavailability of polyphenols in the upper gastrointestinal tract is quite lower, and large amounts of polyphenol accumulate in the colon [64]. Gut microbiota can produce multiple enzymes, such as β -glucuronidase, β -glucosidase, hydrogenases, dehydroxylase, esterases and demethylase, to break down the glucuronides, glycosides, sulfates, esters and lactones [65]. Consequently, polyphenols with complex structures are degraded by the microbiota to form lots of simple structures and alter gut microbiota composition. Moreover, intake of MAC and polyphenols from fruits and vegetables have posed their protective effects against IBD via regulating IBD-associated gut microbiota. For example, administration of pterostilbene and resveratrol, which are two polyphenols found in various berry fruits, in the chemically induced colitic mice increased the fecal

abundance of *Bifidobacterium* and decreased the fecal abundance of *Bilophila* [31, 66]. MAC from various types of fruits and vegetables also displayed protective effects against IBD via alteration of gut microbiota composition [67, 68].

Murine models have been successfully established to evaluate the effects of oral administration of bioactive compounds on IBD in recent years. Colitis induced by chemicals, namely dextran sodium sulfate (DSS), trinitrobenzene sulfonic acid (TNBS) and acetic acid, and genetic technologies, such as interleukin-10 gene-deficient (IL-10^{-/-}), T cell receptor (TCR) mutant, and multi-drug resistant (Mdr1a), have been used for modeling IBD [69]. Furthermore, the severity of the IBD can be determined by multiple endpoints, such as histological damage, proinflammatory cytokines, intercellular adhesion molecules, and gut microbiota [70]. Numerous studies indicated that edible terrestrial plant-based foods, especially fruits and vegetables, have been found to exert inhibitory properties on the IBD based on these endpoints. For example, supplementation of red raspberry (5%, w/w), goji berry (3%, w/w), strawberry (5% w/w), cranberry (1.5% w/w) and mushroom (3%, w/w) in diet protected against IBD in mice by repairing colon histological damage, restoring immune homeostasis, reducing the overproduction of proinflammatory cytokines, alleviating the gut microbiota dysbiosis and their metabolite profiles [29, 30, 61, 71-73].

1.2. Bioactive compounds from seaweeds against IBD

Seaweeds are rich in macronutrients and micronutrients that pose protective effects on inflammation. Certain immortalized cell lines, such as Caco-2, IPEC-1, and macrophages, are used to exam the protective effects of bioactive compounds on the intestinal epithelium's barrier function, the production of cytokines and mucosal immune response

in IBD [74]. Polysaccharides, polyphenols and other compounds from seaweeds have been reported to exert their anti-inflammatory capacities in macrophages via regulating proinflammatory cytokines and enzymes [75-77]. In particular, an *in vitro* model of co-culture of Caco-2 and macrophages was established to determine the protective properties of fucoidan from *Laminaria japonica* on colonic inflammation, where fucoidan inhibited the IL-8 gene expression level in epithelial cells and the production of TNF- α in macrophages [78]. In addition, four aqueous extract from *L. japonica* have been shown to enhance the function of intestinal barriers, reduce the permeability of monolayer, increase the production of the tight junction related proteins and decrease the production of nitric oxide and IL-6 in LPS-stimulated Caco-2 cells [79]. These *in vitro* cell studies provided the foundation that seaweed bioactive compounds could play a vital protective role in IBD.

In recent years, an increasing number of animal studies have examined the beneficial effects of bioactive compounds from seaweed in IBD, which are listed in Table.1. Most of these studies are focused on the polysaccharides isolated from the genus of *Gracilaria*, *Eucheuma*, *Ulva*, *Sargassum* and *Caulerpa*. Overall, these bioactive compounds from seaweeds attenuated IBD mainly via restoring colonic damage, repairing immune homeostasis, and reducing the excessive production of proinflammatory cytokines. More importantly, synergistic protective effects of bioactive compounds and probiotics on IBD have been reported. Aqueous extracts from *Laminaria japonica* (0.03%, w/w) alone, or combined with probiotics (0.03%, w/w), including *Bifidobacterium*, *Lactobacillus*, and *Streptococcus*, have been reported to against colitis in DSS-treated mice via repairing colonic damage and restoring immune homeostasis [25]. Further studies have indicated that the protective effects of bioactive compounds from seaweeds on IBD is closely related to their

modification of gut microbiota dysbiosis. For example, the ethanol extract from *Porphyra tenera*, a popular edible red seaweed, produced protective effects against IBD, and altered the abundance of *Clostridium XIVb*, *Alistipes* and *Lactobacillus* in colitis mice [80]. Moreover, the polysaccharides from *Gracilaria fisheri* and *Gracilaria lemaneiformis* ameliorated colitis, altered the abundance of *Enterobacteriaceae*, *Enterorhabdus*, *Desulfovibrio*, *Alistipes*, *Lachnospiraceae* and *Lactobacillus*, and regulated the production of SCFAs in colitis mice [81-84]. Nevertheless, research focused on bioactive compounds from seaweeds against IBD via altering gut microbiota dysbiosis is still scattered, compared with terrestrial plants. In addition, there is still lack of evidence to reveal the role of whole seaweed in IBD. Finally, there is currently no evidence to support a direct role of gut microbiota in mediating the anti-inflammatory effects of seaweeds in IBD.

Table.1. Impact of seaweed extracts against IBD in in vivo studies

Species	Extract	Animal model	Change in biomarkers	References	
Brown sea-weed	<i>Cladosiphon okamuranus Tokida</i>	Fucoidan extracts	DSS-induced colitis	Disease activity index scores↓. Activity of MPO↓. Production of IFN- γ , IL-6↓. Production of IL-10, TGF β ↑.	[85]
	<i>Dictyopteris undulata</i>	Zonarol	DSS-induced colitis	Disease activity index scores↓. Infiltration of immune cell infiltration↓. Production of iNOS, TNF- α , IL-6↓.	[86]
	<i>Fucus vesiculosus</i>	Fucoidan extracts	DSS-induced colitis	Disease activity index scores↓ Histological scores↓. Infiltration of immune cell↓. Production of IL-1a, IL-1 β , IL-10, MIP-1a, MIP-1 β , G-CSF and GM-CSF↓.	[87]
	<i>Laminaria japonica</i>	Aqueous extracts with prebiotics	DSS-induced colitis	Colon length and microscopic scores↑. Histological scores↓. Production of IFN- γ , IL-1 β , IL-6, IL-10, IL-12, IL-12, IL-17, TNF- α ↓.	[25]
	<i>Sargassum muticum</i>	Fucoanthin-Rich extracts	DSS-induced colitis	Disease activity index scores↓. Production of NO, MDA, TNF- α , IL-6↓.	[88]
	<i>Sargassum muticum</i>	Fucoanthin-Rich extracts	AOM-DSS induced colitis-associated colon cancer	Disease activity index scores↓. Production of SOD↓. Proliferation of lymphocyte↑. Production of NO, MDA, TNF- α , IL-6↓.	[88]
	<i>Saragassum fusiforme</i>	Cellulose nanofiber	DSS-induced colitis	Disease activity index scores↓. Production of MPO and NF- κ B↓.	[89]
	<i>Cystoseira usneoides</i>	Algae meroterpene 11-hydroxy-11-	DSS-induced colitis	Body weight loss, colon length shortening↓. histological scores↓. Production of MPO↓.	[90]

		O-methylamentadione		Production of TNF- α , IL-1 β , IL-10, iNOS and COX-2 \downarrow .	
	<i>Turbinaria ornata</i>	Ethanol extracts	DSS-induced colitis	Body weight loss, disease activity scores \downarrow Production of MPO \downarrow .	[91]
Red sea-weed	<i>Eucheuma cottonii</i>	Ethanol extracts	DSS-induced colitis	Production of COX-2, TNF- α , FOXP3, p-STAT3, IFN- γ , IL-4, IL-6, IL-10, IL-17, IL-12 \downarrow . Body weight loss, colon weight loss, colon length shortening \downarrow . Disease activity index scores \downarrow . Histological scores \downarrow .	[92]
	<i>Eucheuma cottonii</i>	Polysaccharides-rich extracts	DSS-induced colitis	Production TNF- α , IL-1 β , IL-6, IL-10 \downarrow . Body weight loss, colon weight loss \downarrow . Disease activity index scores \downarrow . Histological scores \downarrow .	[93]
	<i>Hypnea musciformis</i>	Sulfated polysaccharide	TNBS-induced colitis	Production of TNF- α , IL-1 β , IL-6, IL-10 \downarrow . Colon weight loss and bowel damage \downarrow . Histological scores \downarrow .	[94]
	<i>Gracilaria birdiae</i>	Sulfated polysaccharide	TNBS-induced colitis	Production of TNF- α , IL-1 β \downarrow . Production of glutathione, MDA, MPO and NO ₃ /NO ₂ \downarrow . Intestinal damage \downarrow .	[95]
	<i>Gracilaria candata</i>	Sulfated polysaccharide	Acetic acid-induced colitis	Production of TNF- α , IL-6, MDA, NO ₃ /NO ₂ , MPO \downarrow . Disease activity index scores \downarrow .	[96]
	<i>Gracilaria lemaneiformis</i>	Sulfated polysaccharide	DSS-induced colitis	Production of NO ₃ /NO ₂ , TNF- α , IL-1 β , MPO and GSH \downarrow . Thymus index and colon length \uparrow . Spleen index \downarrow .	[97]
					Production of MPO, ET, TNF- α , IL-6, IL-1 β \downarrow .

	<i>Gracilaria lemaneiformis</i>	oligosaccharides	DSS-induced colitis	Disease activity index scores↓. Production of TNF- α , IL-1 β , IL-6, CCL25, CCR9↓. Abundance of <i>Lachnospiraceae NK4A136</i> and <i>Lactobacillus</i> ↑ Production of SCFAs↑.	[82]
	<i>Gracilaria lemaneiformis</i>	Polysaccharides	DSS-induced colitis	Disease activity index scores↓. Kidney index and liver index↑, and spleen index↓. Abundance of Bacteroidetes↑. The ratio of Firmicutes/Bacteroidetes↓. Abundance of <i>Lachnospiraceae NK4A136</i> and <i>Lactobacillus</i> ↑. Production of SCFAs↑.	[84]
	<i>Gracilaria lemaneiformis</i>	Sulfated polysaccharide	DSS-induced colitis	Colon weight loss↓. Production of TNF- α , IL-1 β , IL-6↓. Production of MUC-2, ZO-1 and claudin-1↑. Abundance of Actinobacteria↑.	[83]
	<i>Gracilaria fisheri</i>	Oligosaccharide	Acetic acid-induced colitis	Disease activity index scores↓. Body weight loss, colon length shortening↓. Abundance of Enterobacteria↓. Production of SCFAs↑.	[81]
	<i>Lithothamnion corallicoides</i>	Mineral extract	IL-10 ^{-/-} induced colitis	Disease activity index scores↓. Production of MPO, SAA, IL-1 β ↓.	[98]
	<i>Porphyra tenera</i>	Ethanol extract	DSS-induced colitis	Disease activity index scores↓. Production of COX-2, TNF- α , IL-6, IL-1 β ↓. Abundance of <i>Clostridium_XIVb</i> ↓, <i>Alistipes</i> and <i>Lactobacillus</i> ↑	[80]
Green seaweed	<i>Blidingia minima</i>	Polysaccharides-rich extracts	DSS-induced colitis	Disease activity index scores↓. Production of pAKT, I κ B- α , NF- κ B, TNF- α , IL-10, IL-1 β , ZO-1, occludin, claudin-1, ET-1, MPO and EPO↓.	[99]

			Activity of DAO↑.		
	<i>Caulerpa mexicana</i>	Methanol extracts	DSS-induced colitis	Body weight loss and disease activity index scores↓.	[26]
	<i>Caulerpa racemosa</i>	Caulerpin	DSS-induced colitis	Production of IFN- γ , IL-17, IL-12, TNF- α , IL-6↓. Disease activity index scores↓. Colon length shortening and damage↓. Production of TNF- α , IFN- γ , IL-6, IL-17, and NF- κ B p65↓. Production of IL-10↑.	[100]
	<i>Ulva linza</i>	Polyphenol-rich extracts	TNBS-induced colitis	Colon length shortening↓. Production of TNF- α , IL-6↓.	[101]
	<i>Ulva lactuca</i>	Selenium nanoparticles decorated polysaccharides	DSS-induced colitis	Disease activity index score↓. Colon length shortening↓. Production of MDA, GSH, GPx, TNF- α , IL-6↓. Infiltration of immune cell↓.	[102]
	<i>Ulva pertusa</i>	Ulvan	DSS-induced colitis	Disease activity index scores↓. Colon length shortening and damage↓. Production of IL-1 β , IL-4, IFN- γ , MDA, GPx, CAT, ZO-1↓.	[103]
	<i>Ulva pertusa</i>	selenized polysaccharides	DSS-induced colitis	Disease activity index scores↓. Production of ZO-1, MDA, CAT, SOD, TNF- α , IL-6, IL-1 β , IL-4↓.	[104]
Other sea-weed	<i>Chlamydomonas debaryana</i>	Oxylipin containing lyophilised biomass & major	TNBS-induced colitis	Body weight loss, colon length shortening, colon damage↓. Production of mucus↑. Production of iNOS, COX-2, TNF- α ↓. Infiltration of neutrophil↓.	[24]

<i>Spirulina platensis</i>	oxylipin con- stituent hydroalco- holic extract	DSS-induced colitis	Disease activity index scores↓. Production of TNF- α , IL-6, MPO, ESR↓.	[105]
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1.3. Seaweed modulates IBD-associated gut microbiome

Gut microbiota homeostasis plays an important role in promoting host health. The imbalance of gut microbiota may trigger certain diseases, including IBD [106, 107]. Bacteria from the phyla of Firmicutes and Bacteroidetes represent more than 90% of gut microbiota. The ratio between these two phyla (the Firmicutes/Bacteroidetes (F/B) ratio) plays a vital in maintaining host health. Accurately, a lower ratio of F/B has been observed in IBD patients [108, 109]. Oral administration of polysaccharides isolated from *Porphyra haitanensis* and *Ulva prolifera* at the dose of 250 mg/kg for 2 weeks in mice slightly reduced the abundance of Firmicutes and elevated the abundance of Bacteroidetes in fecal [110]. Daily consumption of the brown seaweed *Ecklonia radiata* (5%, w/w) and its polysaccharides (5%, w/w) for 1 week in rats significantly decreased the ratio of fecal abundance F/B, and altered the production of SCFAs [111]. Moreover, similar pattern was obtained from rats received the dried powder of *Laminaria japonica* and *Undaria pinnatifida* (10%, w/w) for four weeks [112].

Shifts of specific taxonomic have also been associated with the development of IBD. The probiotic strains of *Lactobacillus* and *Bifidobacterium* posed anti-inflammatory effects via alteration of the inflammation-related cell-signaling pathway. The presence of these bacterial strains in the gut may enhance host defense against IBD [113]. Moreover, recent studies identified that oral gavage of these bacterial alleviated the signs of colitis in mice [114-117]. An *in vitro* fermentation study indicated that sulfated polysaccharides from *Laminaria Japonica* slightly increased the proportion of *Lactobacillus* and *Bifidobacterium* in 48 hours [118]. Administration of probiotics, including *Bifidobacterium*, *Lactobacillus*, and *Streptococcus*, with aqueous extraction of *Laminaria japonica* for 6 weeks

significantly increased the abundance of *Bifidobacterium* in human feces [119]. Furthermore, the abundance of *Clostridium* and *E.coli* was positively associated with IBD [120, 121]. Administration of dried powder of *Laminaria japonica* and *Undaria pinnatifida* (10%, w/w) for four weeks enhanced the abundance of *Prevotella*, *Alistipes*, and *Bacteroides*, reduced the abundance of *Clostridium* and *Escherichia* in rat feces, accompanied with the alteration of the production of SCFAs [112].

Bioactive compounds from seaweeds have been shown to alter the members of Bacteroidetes (including *Bacteroides* and *Prevotella*) and the member of Firmicutes (including *Clostridium*, *Ruminococcaceae*, *Lactobacillus* and *Lachnospiraceae*) in *in vitro* and *in vivo* studies [110, 122-124]. These findings suggest that bioactive compounds from seaweeds can regulate the gut microbiota related to IBD, potentially exerting protective effects on IBD. Nonetheless, the exact roles of seaweeds on gut microbiota in colitis largely remain unknown. In addition, few reports have examined the effects of bioactive compounds from seaweeds on the composition of the gut microbiome in colitis in animal models. But an increasing number of studies elucidated those bioactive compounds from seaweeds modulated the composition and diversity of IBD-associated bacteria and their metabolite profiles in *in vitro* (Table 2) and *in vivo* (Table 3) studies, particularly in the phyla of Firmicutes and Bacteroidetes, and the production of SCFAs. It is noteworthy that most of the compounds used in *in vitro* and *in vivo* studies are polysaccharides. The role of other seaweed compounds, such as polyphenols, on gut microbiota composition is unclear, unlike terrestrial plants, especially for fruits and vegetables, which are more extensively studied [125, 126]. Further research is warranted to obtain a more incisive understanding of the impact of seaweed polyphenols and other bioactive components on gut microbiota and IBD.

Table.2. Impact of bioactive compounds from seaweed on gut microbiota in in vitro studies

Seaweed	Substrate	Dose	<i>In vitro</i> Digestion	Experimental Parameters	Microbial Changes	Metabolite Changes	References
Unknown (Commercial)	Alginate	5 mg/mL	Digestibility unknown	10% (w/v) pooled inoculum	<i>Bacteroides</i> ↑	Total SCFA↑ Propionate↑ Butyrate↑	[122]
Unknown (Commercial)	Carrageenan	1% (w/v)	Non-digestible	20% (w/v) pooled inoculum	<i>Bifidobacteria</i> ↑ <i>Prevotella</i> ↑ <i>Megamonas</i> ↑ (KO3, KO6) <i>Streptococcus</i> ↑ <i>Lactobacillus</i> ↑ (KO3) <i>Megasphaera</i> ↑ (KO6)	Total SCFA ↑ (KO3) Total SCFA↓ (KO6)	[127]
Unknown (Commercial)	Laminarin	1% (w/v)	Digestibility unknown	10% (w/v) pooled inoculum	<i>Bifidobacteria</i> ↑ <i>Bacteroides</i> ↑	Total SCFA↑ Acetate↑ Propionate↑	[123]
Unknown (hand-made)	Neoagaro-oligosaccharides (NAOS)	1% (w/v)	Non-digestible	1% (w/v) of beneficial bacteria	<i>Lactobacillus</i> ↑ <i>Bifidobacterium</i> ↑	Not provided	[128]
Unknown (commercial)	Porphyran	1% (w/v)	Digestibility unknown	10% (w/v) pooled inoculum	<i>Bifidobacteria</i> ↑ <i>Bacteroides</i> ↑	Not significant difference	[123]
Unknown (commercial)	Ulvan	1% (w/v)	Digestibility unknown	10% (w/v) pooled inoculum	<i>Bifidobacteria</i> ↑ <i>Bacteroides</i> ↑	Acetate↑ Lactate↑	[123]
<i>Ascophyllum nodosum</i>	Sulphated polysaccharide extract	9 mg/mL	Non-digestible	10% (w/v) pooled inoculum	<i>Bacteroides</i> ↑ <i>Phascolarctobacterium</i> ↑ <i>Oscillospira</i> ↑ <i>Fecalibacterium</i> ↑	Total SCFA↑ Acetate↑ Propionate↑ Butyrate↑	[129]
<i>Ecklonia radiata</i>	Crude fraction (CF) High-molecular weight	1.5% (w/v)	71.5% (CF) 0% (HPF) 86.1% (LPF) 87.3% (PF)	10% (w/v) pooled inoculum	<i>Lactobacillus</i> ↑ <i>Bifidobacterium</i> ↑ (LPF) Firmicutes↑ <i>F. prausnitzii</i> ↑	Total SCFA↑ (CF, LPF, HPF) Acetate↑ (CF) Propionate↑	[130]

	polysaccharide fraction (HPF)				<i>C. coccoides</i> ↑ (CF, LPF)	(CF, LPF, HPF)	
	Low-molecular weight polysaccharide fraction (LPF)				<i>Enterococcus</i> ↓ (CF, PF) Bacteroidetes ↑ <i>E. coli</i> ↑ (CF, PF, LPF, HPF)	Butyrate ↑ (CF, LPF, HPF)	
	Phlorotannin-enriched fraction (PF)						
<i>Ecklonia radiata</i>	Water extract (WE)	1.5% (w/v)	Digestibility unknown	10% (w/v) pooled inoculum	<i>Bifidobacterium</i> ↑ <i>Lactobacillus</i> ↑ <i>Bacteroidetes</i> ↑ <i>C. coccoides</i> ↑ (CEE) <i>E. coli</i> ↑ <i>Enterococcus</i> ↑ (WE, AE, CEE, AEE, FF, PF, SP) Total bacteria ↑ (CEE, AEE, WE, FF)	Total SCFA ↑ Acetate ↑ Propionate ↑ Butyrate ↑ (WE, AE, CEE, AEE, FF, PF, SR)	[131]
	Acid extract (AE)						
	Celluclast enzyme extract (CEE)						
	Alcalase enzyme extract (AEE)						
	Free sugar fraction (FF)						
	Polysaccharide fraction (PF)						
	Seaweed powder (SP)						
	Seaweed residue (SR)						
<i>Gracilaria lemaneiformis</i>	sulfated polysaccharides (GLP)	1% (w/v)	Digestibility unknown	10% (w/v) single inoculum	Bacteroidetes ↑ Firmicutes ↑ <i>Escherichia</i> ↓	Total SCFA ↑	[132]
	agaro-oligosaccharides (GLO)	1% (w/v)	Digestibility unknown	10% (w/v) single inoculum	Firmicutes ↑ Actinobacteria ↑	Total SCFA ↑	[132]

<i>Gracilaria rubra</i>	Polysaccharide extract (PE)	1% (w/v)	Non-digestible	10% (w/v) pooled inoculum	Bacteroidetes↑ Firmicutes↓ <i>Prevotella</i> ↑ <i>Phascolarctobacterium</i> ↑	Total SCFA↑ Acetate↑ Propionate↑ Isobutyrate↑	[124]
<i>Laminaria japonica</i>	sulfated polysaccharides	1% (w/v)	Non-digestible	10% (w/v) pooled inoculum	<i>Lactobacillus</i> ↑ <i>Bifidobacterium</i> ↑	Total SCFA↑ Butyrate↑ Acetate↑ Lactic acid↑	[118]
<i>Laminaria japonica</i>	Alginate	0.5% (w/v)	Non-digestible	20% (w/v) pooled inoculum	<i>Faecalibacterium</i> ↑ <i>Bacteroides</i> ↑	Total SCFA↑	[133]
<i>Kappaphycus alvarezii</i>	Whole Seaweed (WS)	1% (w/v)	Non-digestible	10% (w/v) single inoculum	<i>Bifidobacterium</i> ↑ <i>Clostridium histolyticum</i> ↓ <i>Clostridium coccooides</i> / <i>Eubacterium rectale</i> ↓	Total SCFA ↑	[134]
<i>Porphyra haitanensis</i>	Polysaccharide extract	1% (w/v)	Digestibility unknown	10% (w/v) single inoculum	Firmicutes↑ Bacteroidetes↑ Proteobacteria↑	Total SCFA↑ Acetate↑ Propionate↑ Butyrate↑	[135]
<i>Sargassum fusiforme</i>	Polysaccharide extract	20% (w/v)	Digestibility unknown	20% (w/v) pooled inoculum	<i>Faecalibacterium</i> ↑ <i>Bifidobacterium</i> ↑ <i>Ruminococcaceae</i> ↑ <i>Lactobacillus</i> ↑ <i>Prevotella</i> ↓ <i>Blautia</i> ↓	Total SCFA↑ Acetate↑ Propionate↑ Butyrate↑ Valerate↑	[136]
<i>Sargassum thunbergii</i>	Polysaccharide extract	0.3% (w/v)	Digestibility unknown	20% (w/v) pooled inoculum	Bacteroidetes↑ Firmicutes↓ <i>Bifidobacterium</i> ↑ <i>Roseburia</i> ↑ <i>Parasutterella</i> ↑ <i>Fusicatenibacter</i> ↑ <i>Coprococcus</i> ↑ <i>Fecalibacterium</i> ↑	Total SCFA↑ Acetate↑ Propionate↑ Butyrate↑ Valerate↑	[137]
<i>Sargassum muticum</i>	<i>Sargassum muticum</i> Alcalase enzyme extract	1% (w/v)	Digestibility unknown	10% (w/v) single inoculum	<i>Bacteroides/Prevotella</i> ↑ <i>C.coccooides/E.rectale</i> ↓	Total SCFA↑	[138]

(SAE)

Table.3. Impact of bioactive compounds from seaweed on gut microbiota in in vivo studies

Seaweeds	Substrates	Model	Changes in microbiota	Changes in metabolites	References
Unknown	Sodium alginate (Commercial)	Wistar male rats (cecum)	Bacteroides ↑ Presence of <i>Enterorhabdus</i>	Not provided	[139]
<i>Eisenia bicyclis</i>	Laminarin (Commercial)	Wistar male rats (cecum)	Proteobacteria↑ Presence of <i>Lachnospiracea</i> ,	Not provided	[139]
Unknown	Agarose (Neoagarotetraose)	Male Balb/c mice (feces)	<i>Prevotella</i> ↑ <i>Bacteroides</i> ↑ Firmicutes↑ <i>Bifidobacterium</i> ↑ <i>Lactobacillus</i> ↑ <i>Clostridium</i> ↑	Total SCFA↑	[140]
Unknown	Neoagaro-oligosaccharides (NAOS)	Female kunming mice	<i>Lactobacillus</i> ↑ <i>Bifidobacterium</i> ↑	Not provided	[128]
Unknown	Laminarin (Commercial)	male Wistar rats (cecum)	<i>Clostridium ramosum</i> ↑ <i>Parabacteroides distasonis</i> ↑	Total SCFA↑ propionate↑ indole↓ phenol↓ H ₂ S↓	[141]
Unknown	Sodium alginate (Commercial)	male Wistar rats (cecum)	<i>Bacteroides capillosus</i> ↑	Total SCFAs↑ indole↓ phenol↓ H ₂ S↓	[141]
Unknown	Laminarin (Commercial)	female BALB/C mice with high-fat diet (feces)	<i>Parabacteroides</i> ↑ <i>Bacteroides</i> ↑ <i>Clostridium XIVa</i> ↓ <i>Clostridiaceae</i> ↓ <i>Ruminococcus</i> ↑ <i>Roseburia</i> ↑ <i>Lachnospira</i> ↑ <i>Blautia</i> ↓	Not provided	[142]
Unknown	Sodium alginate	Pigs (feces)	<i>Lactobacillus</i> ↑ <i>Talassospira</i> ↑ <i>Anaeroplasm</i> ↑	Not provided	[143]
<i>Ascophyllum nodosum</i>	Fucoidan extracts	Male C57BL/6 mice (cecum)	<i>Lactobacillus</i> ↑ <i>Talassospira</i> ↑ <i>Anaeroplasm</i> ↑	lipopolysaccharide-binding protein in serum↓	[144]

			<i>Candidatus arthromitus</i> ↓ <i>Pep- tocooccus</i> ↓ Lachnospiraceae incertae sedis↓		
<i>Ecklonia cava</i>	Whole seaweed	Weaning pigs (cecal)	<i>Lactobacillus</i> ↑ <i>E. coli</i> ↓	Not provided	[145]
<i>Ecklonia ra- diata</i>	Whole seaweed (WS) & Polysac- charides fraction (PF)	male Sprague-Dawley rats (ce- cum)	<i>F. Prausnitzii</i> ↑ <i>E. coli</i> ↑ (PF) <i>Enterococcus</i> ↓ (WS) <i>Lactobacillus</i> ↓ (PF & WS) <i>Bifidobacterium</i> ↓ (PF & WS) Firmicutes/Bacteroidetes↓ (PF & WS)	Acetate ↑ Propionate ↑ Butyrate ↑ Total SCFA ↑ Phenol ↓ p-cresol ↓ Valerate↓	[111]
<i>Enteromor- pha clathrata</i>	polysaccharides	Male and female C57BL/6J Mice (cecum)	<i>Bifidobacterium spp</i> ↑ <i>Akkermansia muciniphila</i> ↑ <i>Lactobacillus spp</i> ↑ More alteration in male mice	lipopolysaccha- ride-binding pro- tein in female mice↓	[146]
<i>Enteromor- pha prolifera</i>	Whole seaweed (WS & crude poly- saccharides extracts (CPE)	Kunming female mice (feces)	WS: Firmicutes↑ Actinobacteria↑ Bacteroidetes↓ Proteobacteria↓ CPE: Prevotellaceae↑ Firmicutes↑ Actinobacteria↑ Bacteroidetes↓ Proteobacteria↓	Diminished histo- pathological lesions of inflam- matory infiltra- tions and reduced serum levels of nitric ox- ide	[147]
<i>Lami- naria.spp</i>	Laminarin Fucoidan	Pigs (feces)	<i>Lactobacillus</i> ↑	Propionate↓	[148]
<i>Laminaria digitata</i>	Laminarin/Fucoidan Extract (SD) Wet Seaweed (WS)	Pigs (Ileum, Caecum, Colon)	<i>Lactobacillus</i> ↑ <i>Bifidobacterium</i> ↑ (SD) <i>Lactobacillus</i> ↑ <i>Bifidobacterium</i> ↑ (WS)	Not provided	[149]
<i>Laminaria japonica</i>	Fucoidan extracts	Male C57BL/6 mice (cecum)	<i>Ruminococcaceae</i> ↑ <i>Alistipes</i> ↓ <i>Clostridiales</i> ↓	lipopolysaccha- ride-binding pro- tein in serum↓	[144]

<i>Laminaria japonica</i>	Dried powder	female Sprague-Dawley rats (feces)	<i>Akkermansia</i> ↓ Firmicutes/Bacteroidetes↓ <i>Clostridium</i> ↓ <i>Escherichia</i> ↓ <i>Enterobacter</i> ↓	Total SCFA↑ Butyric acid↑ Acetic acid↑ Propionic acids↑	[112]
<i>Lessonia fravicans</i> and <i>L. nigrescens</i> mixture with 2:1	Alginate	Male human	<i>Bifidobacteria</i> ↑ <i>Enterobacteriaceae</i> ↓	Acetic acid↑ Propionic acid↑ Sulphide↓ phenol↓ p-cresol↓ indole↓ ammonia ↓ skatole↓	[150]
<i>Porphyra haitanensis</i>	Polysaccharides	male C57BL/6J mice (feces)	Bacteroidetes↑ Firmicutes↓ <i>Prevotellaceae</i> ↑ <i>Rikenellaceae</i> ↑ <i>Lactobacillus</i> ↓ <i>Lachnospiraceae</i> ↓ <i>Ruminococcaceae</i> ↑	Not provided	[110]
<i>Undaria pin-natifida</i>	Dried powder	female Sprague-Dawley rats (feces)	Firmicutes/Bacteroidetes↓ <i>Clostridium</i> ↓ <i>Escherichia</i> ↓ <i>Enterobacter</i> ↓	Total SCFA↑ Butyric acid↑ Acetic acid↑ Propionic acids↑	[112]
<i>Ulva prolifera</i>	Polysaccharides	male C57BL/6J mice (feces)	Bacteroidetes↑ Firmicutes↓ <i>Lachnospiraceae</i> ↓ <i>Lactobacillus</i> ↓ <i>Prevotellaceae</i> ↑ <i>Rickenellaceae</i> ↑ <i>Ruminococcaceae</i> ↑	Not provided	[110]
<i>Laminaria japonica</i>	Aqueous extract of laminaria japonica with lactic acid bacteria	Human (feces)	<i>Bifidobacterium brevis</i> ↑ <i>Bifidobacterium lactis</i> ↑ <i>Streptococcus thermophilus</i> ↑ <i>Bifidobacterium longum</i> ↑	Not provided	[119]

1.4. Major bioactive compounds versus whole seaweed against IBD

The anti-inflammatory capacities of seaweeds against IBD may be based on (a) isolated bioactive compounds restored colonic damage and repaired immune homeostasis; (b) isolated bioactive compounds altered IBD-associated gut microbiota. As shown in Table.1-3, the major compounds from seaweeds assessed in previous studies were polysaccharides. Polysaccharides, as one of the major components in seaweeds, make up ~70% of dry weight in seaweeds [151]. The structure, composition, bioavailability, and biological activity of polysaccharides varied among different types of seaweeds. Alginate, fucoidan and laminarin are commonly found in brown seaweeds, agar, carrageenan, and porphyrin are found in red seaweeds, while ulvan is normally detected in green seaweeds [152]. Intake of seaweed polysaccharides has been reported to produce beneficial metabolites, mainly SCFAs, such as butyrate, acetate, and propionate, and gases such as H₂, CO₂, and methane, to promote colon health [151]. These polysaccharides posed their ability to modulate IBD-associated gut microbiota [123, 148, 150], and exerted anti-inflammatory capacities in both *in vitro* and *in vivo* studies [153-155].

Polyphenol is another major isolated bioactive compound present in seaweeds with potential health benefits against IBD [156]. Seaweeds are rich in polyphenols such as catechin, catechin gallate, epicatechin, epicatechin gallate, gallic acid, epigallocatechin gallate, flavonols and phlorotannins [157, 158]. The current understanding of the role of phenolic compounds from seaweeds in human gastrointestinal tract disease is scattered. However, eckol and dieckol, as two representative phlorotannins compounds commonly found in brown seaweed, have been reported to against acute colitis in DSS-treated mice via repairing colon damage and restoring immune homeostasis. Moreover, eckol has also been

reported to alter the abundance of *bifidobacterium*, *lactobacillus* and *enterococcus* in cecal contents [159, 160]. In addition, polyphenol-rich extracts from green seaweed *Ulva Linza* were reported to ameliorate colitis [101]. Also, polyphenols from *Callophyllis japonica* [161], *U. Linza* [101], *P. dentata* [76], and *Ecklonia cava* [162, 163] posed anti-inflammatory capacities in LPS-stimulated macrophages. These results provided evidence to support that seaweed polyphenols have potential health benefits against IBD.

The biological effects of isolated bioactive compounds and whole seaweed may be different. The antagonistic or synergistic effects may be produced when different compounds interact in the whole seaweed. Interestingly, recent studies reported that some isolated bioactive compounds from seaweeds might have immunostimulatory effects. For instance, four purified xylogalactomanans fractions from red seaweed *Caulerpa lentillifera* enhanced phagocytosis, nitrite oxide production and acid phosphatase activity in LPS-stimulated macrophage [164]. As one of the major polysaccharides in brown seaweeds, laminarin also promoted the production of nitrite oxide and various cytokines, such as IL-6, MCP-1, VEGF, in activated macrophages [165]. Furthermore, sulfated polysaccharides from red seaweed *Nemalion helminthoides* significantly stimulated the production of IL-6 and TNF- α in both *in vitro* and *in vivo* studies [166]. These results suggested that some components from certain types of seaweed may promote the development of inflammation. Therefore, it is important to investigate the effects of whole seaweed. The doses of seaweed bioactive compounds used in these studies may be higher than the daily intake amounts from whole seaweed, compared with the fruits and vegetables, which may lead to an overestimation of their immunostimulatory efficacy and the results might not be relevant to the effects of dietary whole seaweed [167]. However, the potential synergistic or antagonistic

effects among different bioactive compounds in whole seaweed were neglected in current studies. So far, very few studies have targeted the effect of whole seaweed in the colonic inflammation-related gut microbiota in animal models. A few *in vitro* studies compared the effects of different fractions from seaweed on human gut microbiota. The different fractions exerted distinctive roles in modulating the gut microbiota composition [130, 131]. Thus, it is necessary to investigate how isolated bioactive compounds and whole seaweed play their role in modulating IBD-associated gut microbiota.

CHAPTER 2

INHIBITORY EFFECTS OF POLYPHENOLS-RICH COMPONENTS FROM THREE EDIBLE SEAWEEDS ON INFLAMMATION AND COLON CANCER IN VITRO

2.1. Introduction

Inflammatory agent is an essential response to harmful stimuli caused by stress, infection and injury and is characterized by symptoms as heat, swelling, redness and pain [168]. Chronic inflammation has a strong association with chronic diseases, including cancer and heart disease [169]. Macrophages stimulated by lipopolysaccharides (LPS) or interferon-gamma excessively secrete proinflammatory cytokines, including interleukin (IL)-1, IL-6 and tumor necrosis factors- α (TNF- α), which in turn induce the expression of proinflammatory enzymes, namely cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) [170]. Studies have indicated that overexpression of these proinflammatory cytokines and enzymes are associated with tumor formation in the brain, breast, lung, colorectal and prostate [171, 172]. Natural bioactive compounds from terrestrial plants have been reported to offer beneficial effects against chronic disease. The application of seaweeds, the largest and most complex algae, as foodstuffs for human health traced back to several hundred years ago in Asian countries, due to the richness of bioactive compounds such as polysaccharides, polyphenols, minerals, fatty acids, bioactive peptides and proteins [19]. Bioactive compounds from seaweeds have been shown to against inflammation and cancer in cell culture and animal studies [76, 101, 173].

Polyphenols are secondary metabolites from plant which may offer health benefits against chronic diseases, such as oxidative stress, inflammation, cancer [174]. Polyphenols can be divided into two categories during the process of extraction. Extractable polyphenols which can be acquired by aqueous organic solvent, and non-extractable polyphenols which remained in the residues and largely ignored in most prior studies [175]. Non-extractable polyphenols includes low molecular weight polyphenols (phenolic acids and flavonoids) cross-linking with dietary fiber and proteins, and macromolecules polyphenols (condensed tannins and proanthocyanidins) [176]. Non-extractable polyphenols compounds isolated from fruits and vegetables have been reported with anti-oxidative, anti-inflammatory and anti-cancer properties [177-180].

To date, multiple phenolics compounds have been isolated and quantified from edible seaweeds and have been reported with various biological properties [76, 161, 181]. These phenolic compounds belong to extractable polyphenols, where the potential health benefits of non-extractable polyphenols from these popular edible seaweeds remain unclear. However, polyphenols compounds from edible seaweed, particularly for those with protective effects on inflammation and colon cancer, have not been adequately investigated. Brown seaweed *Laminaria japonica*, red seaweed *Porphyra tenra* and green seaweed *Ulva lactuca* are three popular edible seaweeds, which are widely distributed in Asian countries and used as a drug in Traditional Chinese Medicine [182-184]. Thus, the current study aims to characterize the compositions of extractable polyphenol-rich components (EPCs) and non-extractable polyphenol-rich components (NEPCs) from *L. japonica*, *P. tenera* and *U. lactuca*, as well as to investigate their anti-colon cancer and anti-inflammatory efficacy and mechanisms.

2.2. Materials and Methods

2.2.1 Materials

Dried powder of *L. japonica* and *U. lactuca* were obtained from Wonderful LLC (Fuzhou, Fujian, China) in January of 2020, dried powder of *P. tenera* was purchased from PlantGift LLC (Haozhou, Anhui, China) in January of 2020. The seaweed powders were stored at -20°C before use. 3-Hydrobenzoic acid, 4-hydrobenzoic acid, ferulic acid, iso-ferulic acid, sinapic acid, phloroglucinol, syringic acid, coumaric acid, rutin, hesperidin, luteolin, rosmamaric acid, apigenin, caffeic acid, gallic acid, chlorogenic acid, vanillic acid, myricetin, morin, quecertin, acacetin, kaempferol, catechin, epicatechin, gallo-catechin, epigallocatechin gallate, epigallocatechin, and epicatechin-gallate were ordered from Shyuanye (Shanghai, China).

2.2.2 Preparation of polyphenols-rich components

The extraction of polyphenols-rich components was conducted based on our previously report with some modifications [178]. Briefly, the dried powders of edible seaweeds were blended with chilled 70% (v/v) acetone aqueous. The blend was subjected to ultrasound vibration for half an hour, before spinning at 3,000g for 10 min. The residues were subjected to the same procedure two times. After that, the supernatant was pooled, concentrated and subjected to the extraction of EPCs and the residues were collected for the extractions of NEPCs.

For the preparations of EPCs, the supernatants were dissolved in methanol. The hexane was used to separate highly lipophilic molecules before the methanol layers were

concentrated, followed by the extraction of ethyl-acetate. Finally, upper layer was pooled, dried, and stored in the freezer for further analysis.

For the preparations of NEPCs, the residues were blended with sodium hydroxide (2M) for 2 hours, where the container were purged with nitrogen. Then, concentrated hydrochloric acid was added to terminate reaction, before spin at 4,000g for 10 min. Subsequently, the supernatant was processed to the extraction of ethyl-acetate. Finally, the upper phase was pooled, dried and stored at -20°C.

2.2.3 Evaluation of total phenolics contents (PCs), flavonoids contents (FCs), tannins contents (TCs), carbohydrates contents (CCs), and proteins content (PRCs)

PCs were measured by Folin–Ciocalteu method as previously studies [185]. A volume of 20 µL of samples or gallic acid solutions (0 to 200 µg/mL) was added into 96-well plate with 20 µL distilled water and 20 µL of Folin-Ciocalteu reagent. The plate was kept at room temperature for 10 min, before adding 140 µL of 7% sodium carbonate. Finally, the plate was stand at room temperature for another 90 min, followed by measuring absorbance at 760 nm using a spectrophotometer (BioTek Instrument, Inc. Winooski, VT, USA), and results were presented as mg of gallic acid equivalents per hundred g seaweed powder (mg GAE/100 g seaweed powder).

FCs was measured by Aluminium trichloride method as previously studies [186]. A volume of 20 µL of samples or catechin solutions (0 to 200 µg/mL) was added into 96-well plate with 10 µL of 5% sodium nitrite and 100 µL distilled water. First, the plate was kept at room temperature for 6 min before adding 20 µL of aluminum chloride. Then, the plate was incubated at room temperature for another 5 min before adding 50 µL of sodium

hydroxide (1M). Finally, the absorbance was monitored at 510 nm using a spectrophotometer (BioTek Instrument), and results were presented as mg of catechin equivalents per hundred g seaweed powder (mg CE/100 g seaweed powder).

TCs were measured by vanillin-sulfuric acid method as previously studies [187]. A volume of 20 μL samples or catechin solutions (0 to 200 $\mu\text{g}/\text{mL}$) was added into 96-well plate mixed with 90 μL of 30% concentrated sulfuric acid and 90 μL of 4% vanillin in methanol, followed by the stand for 5 min at room temperature. Finally, the absorbance was read at 510 nm using a spectrophotometer (BioTek Instrument), and results were presented as mg of catechin equivalents per hundred g seaweed powder (mg CE/100 g seaweed powder).

CCs were assessed by phenol-sulfuric acid method as previously studies [188]. A volume of 50 μL of samples or glucose solutions (0 to 200 $\mu\text{g}/\text{mL}$) was added into 96-well plate, followed by adding 30 μL of 5% phenol and 150 μL of concentrated sulfuric acid rapidly. Finally, the plate was heated at 90°C for 5 min, followed by measuring the absorbance at 490 nm using a spectrophotometer (BioTek Instrument), and results were presented as mg of glucose equivalents per hundred g seaweed powder (mg GE/100 g seaweed powder).

PRCs were evaluated by the BCA method with minor modifications [189]. Results were presented as mg of protein per hundred g seaweed powder (mg protein/100 g seaweed powder).

2.2.4 Evaluation of antioxidant properties of EPCs and NEPCs

ORAC was assessed following previously studies with minor modifications [190]. A volume of 20 μL samples or Trolox solutions (0 to 200 μM) was added into 96-well plate with 40 μL of 75 μM fluorescein solution. The plate was gently shaken and stored at 37°C for 2 min, before adding 140 μL of 0.8 M 2,2'-Azobis (2-amidinopropane) dihydrochloride solution. Finally, the plate was subjected to a microplate fluorescence reader (BioTek Instrument), excitation was measured at 485 nm and emission was measured at 528 nm. This process continued for 2 hours and the absorbance was recorded with an interval of 2 min. Results were presented as μmol of Trolox equivalents per g extract ($\mu\text{mol TE/g}$ extract).

DPPH \cdot scavenging capacity were determined following previously studies with minor modification [191]. A volume of 20 μL of samples or Trolox solutions was added into 96-well plate mixed that 180 μL of 50 μM DPPH \cdot ethanol solution. Finally, the plate was kept at room temperature for half-hour, followed by measuring absorbance at 517 nm by a spectrophotometer (BioTek Instrument), and results were presented as Trolox equivalent antioxidant capacity.

ABTS \cdot scavenging capacity were determined following previously studies with minor modifications [191]. Firstly, the ABTS working solution was prepared by the ratio of 7 mM ABTS solution to 2.45 mM potassium persulfate solution with 1:5. Subsequently, a volume of 10 μL of samples or Trolox solution was added into 96-well plate with 200 μL of ABTS working solution. Finally, the plate was stored at room temperature, avoiding light for 7 min, followed by measuring absorbance at 734 nm using a spectrophotometer (BioTek Instrument), and results were presented as Trolox equivalent antioxidant capacity.

2.2.5 Identification of phenolic compounds

High-resolution LC/MS was performed by an Ultimate 3000 UHPLC system coupled to an Orbitrap Fusion mass spectrometer (Thermo Scientific, Waltham, MA, USA) in the mass spectrometry core facility at the University of Massachusetts Amherst. Chromatography separation was carried out by the reverse-phase Kinetex XB-C18 column (100mm × 4.6 mm, 2.6 μm, Phenomenex, Torrance, CA, USA). Meanwhile, the mobile phase made up of 5% acetonitrile with 0.1% formic acid (solvent A), and 0.1% formic acid in 100% acetonitrile (solvent B). The initial mobile phase composition was 15% solvent B and linearly elevated to 100% solvent B within 3 mins and maintained for 10 mins. Then, the concentration of solvent B was linearly decreased to 15% with 0.01 mins and maintained for 1.99 mins. The flow rate was 400 μL/min, and the injection volume was 5 μL. Data was acquired in positive ESI mode using a spray voltage 3250V, with sheath and aux gas set to 50 and 15 respectively, as well as vaporizer and tube temperature both set to 300°C. Data processing were accomplished using Xcalibur V4.2 (Thermo Scientific).

2.2.6 Cytotoxicity and nitrite oxide assay of RAW 264.7 cells

The cytotoxicity of EPCs and NEPCs on macrophages was tested according to the MTT assay, and the Griess was carried out to investigate nitrite concentration as previously described [192]. Overview, RAW 264.7 cells (5×10^5 cells/mL) were cultured into 96-well plate (200 μL/well) and incubated for 24 h, before treated with or without LPS (1 μg/mL) and coupled with an aliquot of EPCs or NEPCs at multiple concentrations for another 24 h. The cells and the culture media were subjected to MTT assay and Griess reaction, respectively.

2.2.7 Cell viability of normal colon cells and colon cancer cells

The cell viability was performed as we reported previously [193]. CCD-18Co cells (50,000 cells/mL) and HCT116 cells (12,500 cells/mL) were cultured into 96-well plate (200 μ L/well) and incubated at 37 °C overnight, before posed to multiple concentrations of EPCs and NEPCs for another 48 h or 72 h. Finally, the cells were assessed to MTT assay.

2.2.8 Flow cytometer analysis

Flow cytometer analysis were performed as previously study [194]. HCT116 cells (4×10^4 cells/mL) were cultured in 6-well plates and incubated overnight, before posed to EPCs or NEPCs for 24 hours for cell cycle analysis and for 48 hours for cell apoptosis analysis. Then, media containing any floating cells were collected by trypsinization. Finally, cell pellets were washed by chilled PBS and subject to flow cytometer analysis.

2.2.9 qRT-PCR analysis

Total RNA from macrophages was isolated by Trizol reagent. Real-time qRT-PCR assay was carried out as we reported previously [192]. The primer sequence used for cDNA amplification was listed in Table.4. Three independent parallel groups was carried out and related mRNA expression was determined using the $2^{-\Delta\Delta C_t}$ method [195].

Table.4. Primers pairs used for cDNA amplification

Primer	Direction	Sequence	Source
iNOS	Forward	5'-TCC TAC ACC ACA CCA AAC-3'	[192]
	Reverse	5'-CTC CAA TCT CTG CCT ATC C-3'	
COX-2	Forward	5'-CCT CTG CGA TGC TCT TCC-3'	
	Reverse	5'-TCA CAC TTATAC TGG TCA AAT CC-3'	
HO-1	Forward	5'-AAG AGG CTA AGA CCG CCT TC-3'	
	Reverse	5'-GTC GTC GTC AGT CAA CAT GG-3'	
NQO-1	Forward	5'-TCG GAG AAC TTT CAG TAC CC-3'	
	Reverse	5'-TGC AGA GAG TAC ATG GAG CC-3'	
GADPH	Forward	5'-TCA ACG GCA CAG TCA AGG-3'	

TNF- α	Reverse	5'-ACT CCA CGA CAT ACT CAG C-3'	[161]
	Forward	5'-ATG AGC ACA GAA AGC ATG ATC-3'	
IL-1	Reverse	5'-TAC AGG CTT GTC ACT CGA ATT-3'	
	Forward	5'-GAG TGT GGA TCC CAA GCA AT-3'	
IL-6	Reverse	5'-CTC AGT GCA GGC TAT GGA CCA-3'	
	Forward	5'-AGT TGC CTT CTT GGG ACT GA-3'	
	Reverse	5'-CAG AAT TGC CAT TGC ACA AC-3'	

2.2.10 Immunoblotting

The whole-cell protein extraction was based on the previous studies [192]. Briefly, Macrophages were cultured in plates and incubated for 24 h, before posed to multiple concentrations of EPCs or NEPCs for another 24 h. Then, the cell lysate was assessed for immunoblotting. The antibodies of iNOS, COX-2, HO-1 were ordered from Santa Cruz (Dallas, TX, USA), the antibody of β -actin as the loading control was purchased from Sigma-Aldrich.

2.2.11 Statistically analysis

Data were presented as mean \pm standard derivation (SD) of more than three independent parallel experiments. Statistical comparison among groups was performed using One-way ANOVA followed by student's t-test. A p-value <0.05 was considered statistically significant.

2.3. Results and Discussion

2.3.1 Chemical profiles of EPCs and NEPCs in *L. japonica*, *U. lactuca* and *P. tenera*

Seaweed polyphenols have been reported to offer health benefits against oxidative stress, inflammation, cancer [76, 157, 173]. However, most studies into polyphenols only focused in the EPCs. NEPCs, the fraction of polyphenols remaining in the residue after

extraction, were largely neglected by prior studies [175]. Here, we sought to elucidate the compositions of EPCs and NEPCs in three edible seaweeds, *L. japonica*, *U. lactuca* and *P. tenera*, and evaluate their potential protective effects on inflammation and colon cancer in the current study.

The yield of EPCs of *L. japonica*, *U. lactuca* and *P. tenera* were 9.85, 17.26 and 13.27 mg/g dried powder, respectively. The yield of NEPCs of *L. japonica*, *U. lactuca* and *P. tenera* were 15.24, 17.75 and 19.45 mg/g dried powder, respectively. The total PCs, FCs, TCs, CCs and PRCs in EPCs and NEPCs from three edible seaweeds were shown in Figure.2. Interestingly, the PCs, FCs, TCs in NEPCs from *L. japonica* were all higher than those in its EPCs. Similar patterns were observed in *U. lactuca*. Moreover, the PCs and TCs in EPCs were higher than those in its NEPCs in *P. tenera*. The FCs in NEPCs were also higher than those in its EPCs in *P. tenera* (Figure.2). Small amounts of CCs and PRCs (less than 60 mg/100 g dried powder) were identified in EPCs and NEPCs. The relative abundance of PCs in NEPCs and EPCs was comparable to other plant-based foods, such as apples, bananas, carrots, broccoli and lettuce [196]. Also, both EPCs and NEPCs from *L. japonica* and *U. lactuca* contain more tannins than flavonoids.

The selected phenolic compounds in the current study were identified and quantified by UHPLC/MS. Briefly, the abundance of phenolic compounds in the EPCs were higher than those in the NEPCs in three edible seaweeds. But the amounts of phenolic compounds in *L. japonica*, *U. lactuca* and *P. tenera* were different (Table.5). Hydrobenzoic acid, coumaric acid, chlorogenic acid, vanillic acid, caffeic acid, sinapic acid, quercetin, myricetin, catechin, epicatechin, and epigallocatechin gallate were the major constituents in the EPCs. In contrast, iso-ferulic acid, rosmarinic acid, luteolin, acacetin, and

kaempferol were the major constituents in the NEPCs. Overall, the current study offered an incisive understanding of the chemical profiles and biological effects of different bio-active components in *L. japonica*, *U. lactuca* and *P. tenera*. More importantly, for the first time, we characterized the chemical profiles of their NEPCs.

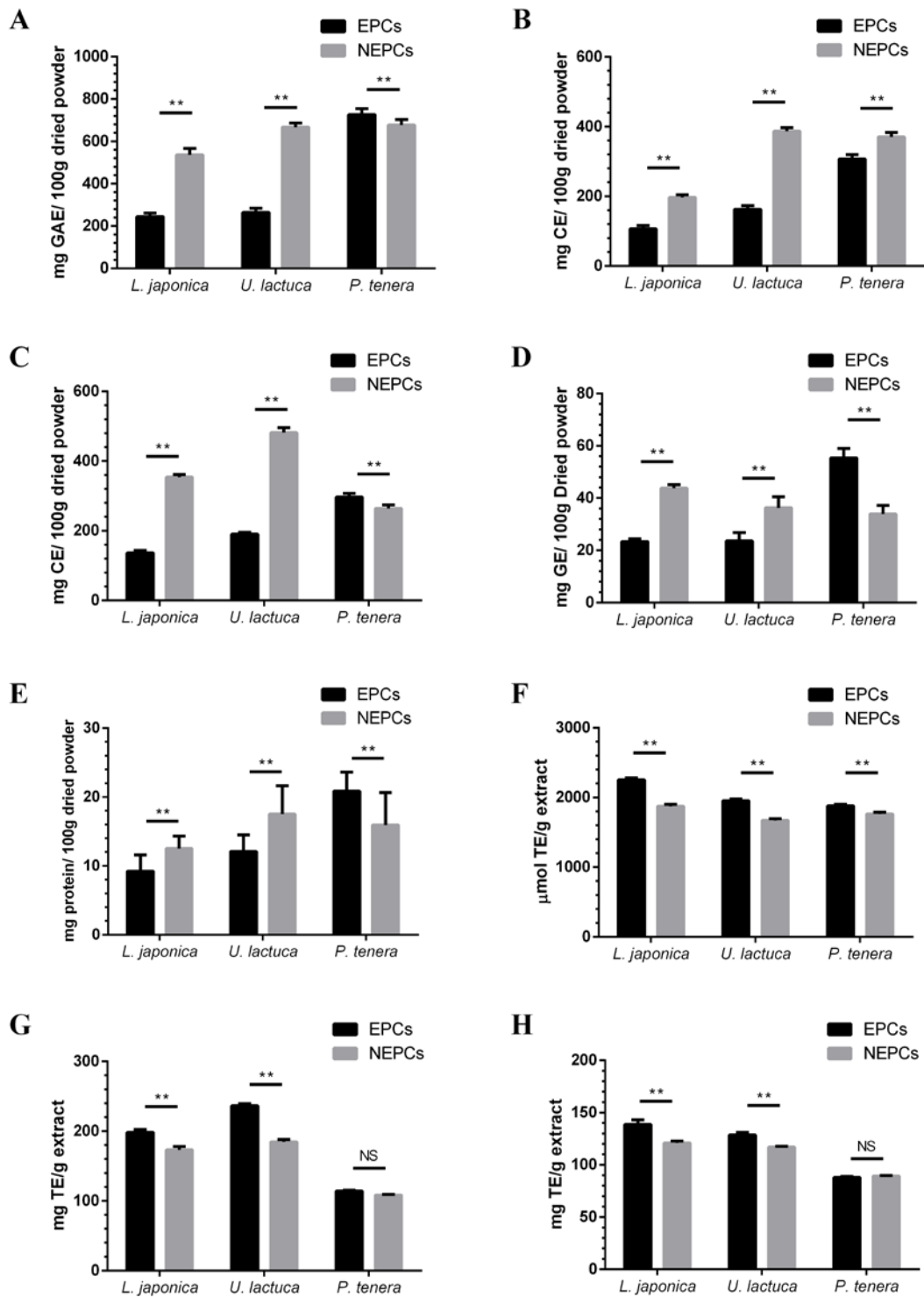


Figure.2. Total phenolic contents (PCs) (A), flavonoid contents (FCs) (B), tannin contents (TCs) (C), carbohydrate content (CCs) (D), protein contents (PRCs) (E) in EPCs and NEPCs from three edible seaweeds. The levels of ORAC (F), DPPH (G) and ABTS (H) of

the EPCs and NEPCs from three edible seaweeds. Data were presented as mean \pm SD (n = 6), ** indicates $p < 0.01$.

Table.5. Phenolic compounds identified in the EPCs and NEPCs in three edible seaweeds.

Compounds	Retention time	MS(m/z)	<i>L. japonica</i>		<i>U. lactuca</i>		<i>P. tenera</i>	
			EPCs (µg/g extract)	NEPCs (µg/g extract)	EPCs (µg/g extract)	NEPCs (µg/g extract)	EPCs (µg/g extract)	NEPCs (µg/g extract)
3-hydrobenzoic acid	3.96	139.039 [M+H] ⁺	770.80 ± 48.92	26.66 ± 2.23	235.27 ± 14.25	23.02 ± 2.24	566.86 ± 50.25	133.70 ± 12.23
4-hydrobenzoic acid	3.15	139.039 [M+H] ⁺	550.59 ± 33.52	ND	781.87 ± 59.82	ND	ND	ND
Phloroglucinol	2.91	127.039 [M+H] ⁺	163.63 ± 15.21	ND	ND	211.60 ± 13.21	814.38 ± 78.82	433.62 ± 41.29
Sinapic acid	4.20	225.076 [M+H] ⁺	530.84 ± 49.89	23.31 ± 2.04	506.95 ± 37.89	3.72 ± 0.92	742.58 ± 67.89	98.11 ± 8.02
Ferulic acid	4.25	195.065 [M+H] ⁺	1175.76 ± 88.13	127.35 ± 2.65	16.32 ± 1.25	36.15 ± 2.52	211.24 ± 18.32	1020.73 ± 92.56
Iso Ferulic acid	4.28	195.065 [M+H] ⁺	ND	17.03 ± 1.52	ND	25.52 ± 1.78	89.69 ± 81.55	1256.29 ± 118.51
Syringic acid	4.33	199.060 [M+H] ⁺	ND	ND	ND	ND	2726.17 ± 182.76	ND
coumaric acid	3.70	165.055 [M+H] ⁺	392.77 ± 4.44	ND	997.78 ± 93.42	ND	1436.86 ± 124.32	17.84 ± 1.84
Rosmarinic acid	4.20	361.092 [M+H] ⁺	1446.66 ± 99.23	244.04 ± 19.21	ND	147.04 ± 12.23	815.44 ± 76.62	1027.65 ± 102.23
Chlorogenic acid	3.47	355.102 [M+H] ⁺	1529.15 ± 111.13	ND	753.70 ± 61.53	191.01 ± 18.11	1928.50 ± 161.25	191.01 ± 18.11
Caffeic acid	3.16	181.050 [M+H] ⁺	628.49 ± 41.22	ND	947.91 ± 81.24	189.36 ± 16.88	1323.60 ± 121.22	193.16 ± 17.89
Vanilic acid	3.15	169.050 [M+H] ⁺	547.75 ± 51.23	8.82 ± 0.87	847.43 ± 31.63	ND	1325.73 ± 71.63	8.82 ± 0.87
Gallic acid	10.98	171.023 [M+H] ⁺	2491.88 ± 216.21	ND	ND	ND	ND	ND
Luteolin	4.52	287.055 [M+H] ⁺	2.39 ± 0.34	221.53 ± 16.54	411.52 ± 39.85	949.12 ± 86.54	408.16 ± 40.12	1074.05 ± 96.54
Rutin	3.88	611.161 [M+H] ⁺	ND	ND	4033.13 ± 378.18	ND	3752.20 ± 368.28	ND
Hesperidin	4.00	611.197 [M+H] ⁺	ND	ND	3120.40 ± 202.13	72.46 ± 6.85	2250.90 ± 202.13	104.55 ± 9.85
Myricetin	4.30	319.045 [M+H] ⁺	897.06 ± 68.89	9.82 ± 0.75	724.98 ± 58.89	1.23 ± 0.07	422.91 ± 28.81	2.82 ± 1.75
Apigenin	4.77	271.060 [M+H] ⁺	100.20 ± 9.13	15.65 ± 1.98	85.12 ± 6.78	33.31 ± 2.98	362.73 ± 26.78	92.51 ± 6.98
Morin	4.44	303.050 [M+H] ⁺	44.30 ± 3.55	5.02 ± 0.51	405.14 ± 3.55	3.42 ± 2.12	1353.32 ± 123.52	1.99 ± 0.12
Quecetin	4.56	303.050 [M+H] ⁺	937.03 ± 92.23	16.99 ± 1.96	1842.13 ± 89.23	5.99 ± 0.96	481.89 ± 29.35	8.99 ± 0.96
Acacetin	5.43	285.076 [M+H] ⁺	ND	909.85 ± 92.97	ND	110.51 ± 9.24	4.91 ± 0.32	201.82 ± 19.24
Kaempferol	4.52	287.056 [M+H] ⁺	3915.07 ± 256.11	213.06 ± 2.55	477.52 ± 36.11	929.76 ± 8.55	ND	988.04 ± 78.34
Catechin	3.70	291.086 [M+H] ⁺	913.19 ± 82.23	1.12 ± 0.03	1926.78 ± 119.23	1.52 ± 0.43	2528.84 ± 231.23	6.22 ± 0.43
Epicatechin	3.86	291.086 [M+H] ⁺	1263.01 ± 102.13	1.78 ± 0.76	737.84 ± 72.13	2.58 ± 0.76	1342.42 ± 132.13	12.58 ± 2.76
Galocatechin	2.97	307.081 [M+H] ⁺	854.06 ± 71.23	1.03 ± 0.16	1348.26 ± 71.23	ND	892.31 ± 16.35	ND
Epigallocatechin gal- late	3.91	459.092 [M+H] ⁺	1525.35 ± 109.12	132.26 ± 11.91	3357.55 ± 209.12	1195.54 ± 101.91	28475.45 ± 254.11	ND
Epigallocatechin	3.15	307.081 [M+H] ⁺	1111.54 ± 96.15	ND	2016.63 ± 154.15	ND	2830.86 ± 211.81	8.18 ± 0.75
Epicatechin gallate	4.06	443.097 [M+H] ⁺	ND	28.26 ± 1.95	397.85 ± 29.12	100.01 ± 12.95	2144.65 ± 121.53	136.59 ± 12.95

Results were expressed as mean ± SD; ND means for not detected.

2.3.2 Antioxidant capacities of the EPCs and NEPCs in *L. japonica*, *U. lactuca* and *P. tenera*

The abundance of phenolics, flavonoids and tannins in EPCs and NEPCs of these seaweeds may have contributed to the antioxidant capacities. The ORAC values were ranging from 1,870 to 2,280 $\mu\text{mol TE/g}$ in EPCs, and from 1,750 to 1,880 $\mu\text{mol TE/g}$ in NEPCs. Furthermore, the ORAC values of both EPCs and NEPCs from *L. japonica* were higher than the *U. lactuca* and *P. tenera* (Fig.2F). The DPPH values were ranging from 110 to 240 mg TE/g in EPCs, and from 100 to 190 mg TE/g in NEPCs (Fig.2G). The ABTS values were ranging from 85 to 140 mg TE/g in EPCs, and from 87 to 122 mg TE/g in NEPCs (Fig.2H). The antioxidant activity of EPCs from *L. japonica* and *U. lactuca* were all significantly higher than the NEPCs measured by the DPPH method and the ABTS method. There was no difference between the activity of EPCs and NEPCs from *P. tenera*. Overall, EPCs and NEPCs from *L. japonica* and *U. lactuca* exhibited stronger antioxidant activities than those from *P. tenera*.

2.3.3 EPCs and NEPCs reduced the NO production in activated macrophages

Epidemiological data has revealed that higher intake of polyphenols might reduce the risk of inflammation [197]. Next, we sought to understand the protective effects of EPCs and NEPCs from three edible seaweeds against inflammation in LPS-treated macrophages. Firstly, their cytotoxicity on RAW264.7 macrophages were monitored by MTT assay at multiple concentrations. Both EPCs and NEPCs from three edible seaweeds did not display any cytotoxicity up to 200 $\mu\text{g/mL}$. Subsequently, these non-toxic ranges were used to evaluate their anti-inflammatory effects in activated macrophages.

NO is a signaling molecule and overproduction of NO during the inflammation process can induce proinflammatory cytokines in macrophages [198]. In the present study, LPS alone significantly stimulated NO production, when compared to the control group. Without LPS stimulation, EPCs or NEPCs from three edible seaweeds did not trigger the overproduction of NO, while they significantly decreased the overproduction of NO stimulated by LPS in a dose-dependent manner. More specifically, the IC₅₀ values of EPCs from *L. japonica*, *U. lactuca* and *P. tenera* were 39.98, 52.43 and 82.43 µg/mL, respectively (Figure.3A, 3B & 3C). The IC₅₀ values of NEPCs from *L. japonica*, *U. lactuca* and *P. tenera* were 69.59, 60.83 and 93.54 µg/mL, respectively (Figure.3A, 3B & 3C). Overall, EPCs had stronger inhibitory effects on NO production in activated macrophages than NEPCs. Also, *L. japonica* and *U. lactuca* showed stronger suppression of the production of NO than *P. tenera*.

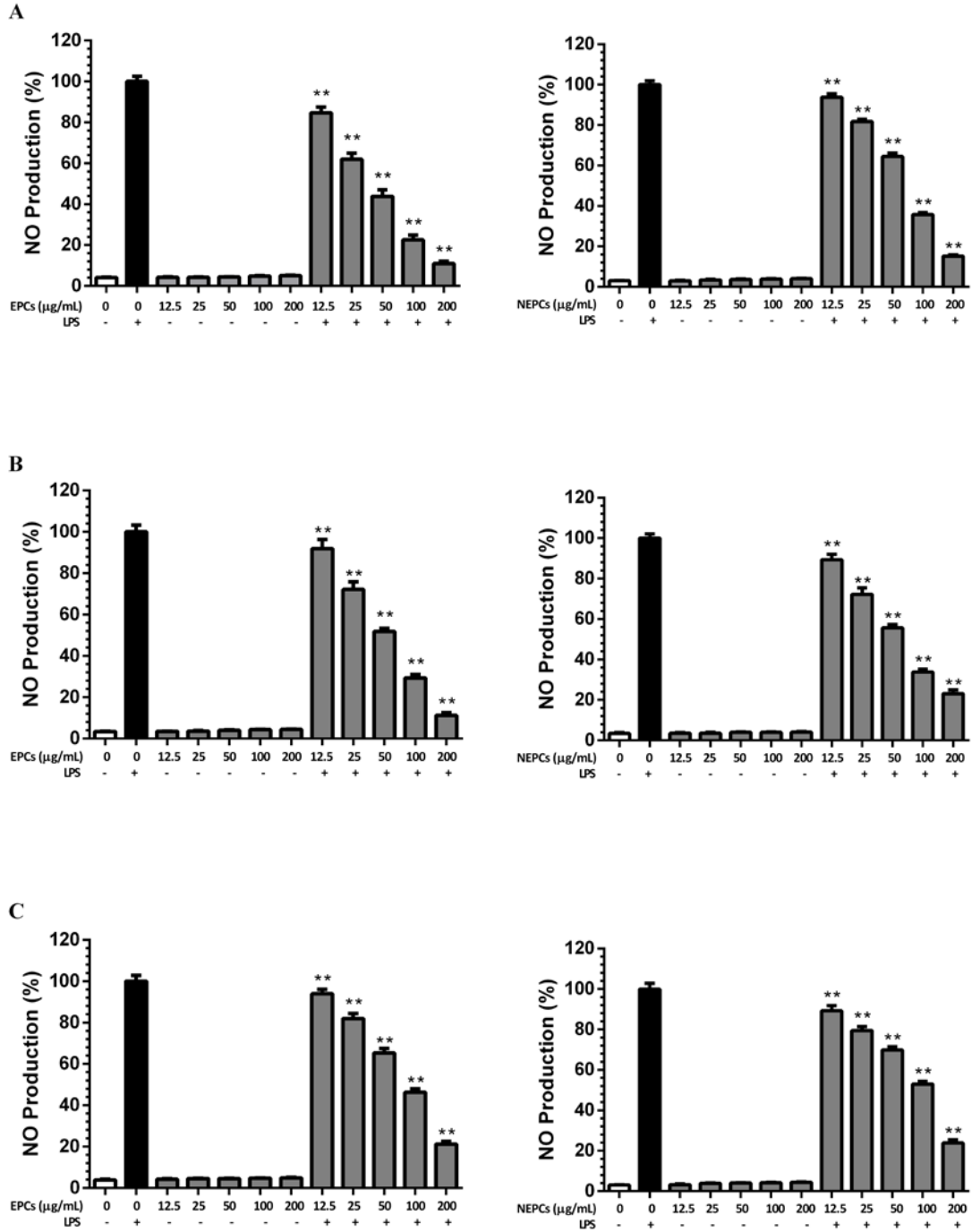


Figure.3. Inhibitory effects of EPCs from *L. japonica* (A), *U. lactuca* (B), *P. tenera* (C) on NO production in activated macrophages. Inhibitory effects of NEPCs from *L. japonica* (D), *U. lactuca* (E), *P. tenera* (F) on NO production in activated macrophages. Results were expressed as mean \pm SD (n = 6). * indicates p < 0.05 and ** indicates p < 0.01, when compared to the LPS group.

2.3.4 EPCs and NEPCs lowered the gene expression of proinflammatory cytokines

LPS stimulation also activates the macrophages to generate pro-inflammatory cytokines [199]. The mRNA expression of IL-1, IL-6 and TNF- α were all slightly raised in response to LPS treatment, and these elevated cytokines were diminished by the treatment of EPCs or NEPCs (Figure.4). EPCs from *L. japonica*, *U. lactuca* and *P. tenera*, at 200 $\mu\text{g/mL}$, suppressed the mRNA expression levels of TNF- α by 74.86, 74.69 and 64.69%, respectively. NEPCs from *L. japonica*, *U. lactuca* and *P. tenera*, at 200 $\mu\text{g/mL}$, reduced the mRNA expression levels of TNF- α by 68.14, 71.85 and 57.96%, respectively (Figure.4A, 4B & 4C). Moreover, similar patterns were observed in the mRNA expression levels of IL-6 and IL-1 (Figure.4). Our results indicated that both EPCs and NEPCs exerted anti-inflammatory effects via suppressing the overproduction of aforementioned cytokines.

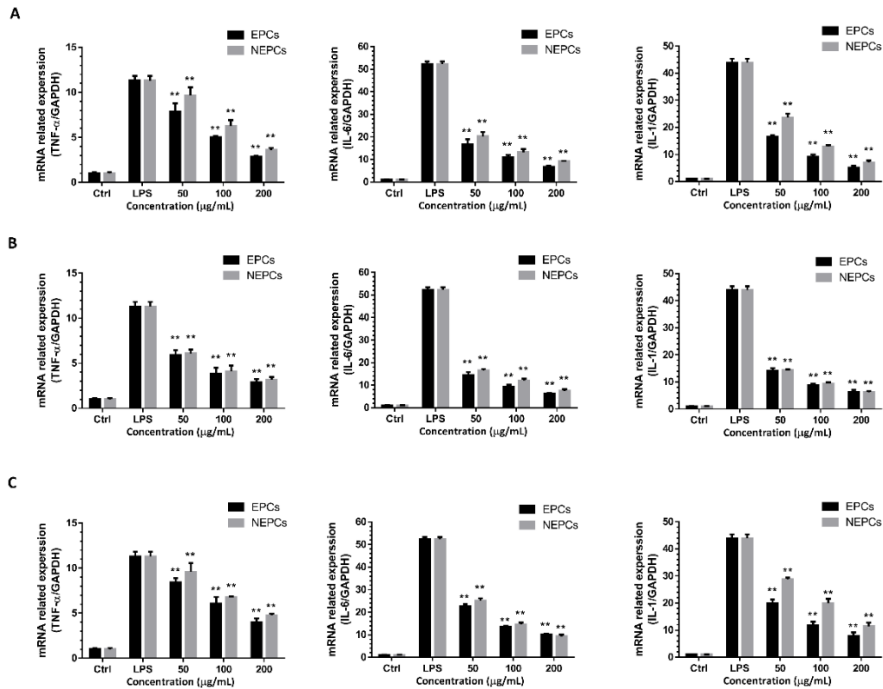


Figure.4. Suppressive effects of EPCs and NEPCs from *L. japonica* (A), *U. lactuca* (B), *P. tenera* (C) on mRNA expression of TNF- α , IL-6, IL-1 in activated macrophages.

Results were expressed as mean \pm SD (n = 6). ** indicates $p < 0.01$ in comparison with the LPS group.

2.3.5 EPCs and NEPCs suppressed iNOS and COX-2 expression in activated macrophages

Pro-inflammatory enzymes, especially for the COX-2 and iNOS, plays a vital role in inflammatory response [200]. The expression of iNOS and COX-2 were greatly elevated in response to LPS stimulation. EPCs and NEPCs from three edible seaweeds lowered their expression (Figure.5 & 6). Specifically, EPCs from *L. japonica*, *U. lactuca* and *P. tenera*, at 200 $\mu\text{g}/\text{mL}$, suppressed the mRNA expression levels of iNOS by 85.89, 88.47 and 72.99%, respectively. NEPCs from *L. japonica*, *U. lactuca* and *P. tenera*, at 200 $\mu\text{g}/\text{mL}$, inhibited the mRNA expression levels of iNOS by 83.96, 85.11 and 67.92%, respectively (Figure.5A, 5B & 5C). Furthermore, the effects of EPCs and NEPCs from three edible seaweeds on the protein expression of iNOS were similar to the mRNA expression levels. EPCs and NEPCs from three edible seaweeds, at 200 $\mu\text{g}/\text{mL}$, reduced the protein expression of iNOS ranging from 52.27 to 95.74% (Figure.6A, 6B & 6C). Similar patterns were acquired in the expression of COX-2 (Figure.5 & 6). These findings suggested that EPCs and NEPCs lowered the production of NO by downregulating iNOS and COX-2 signaling pathways.

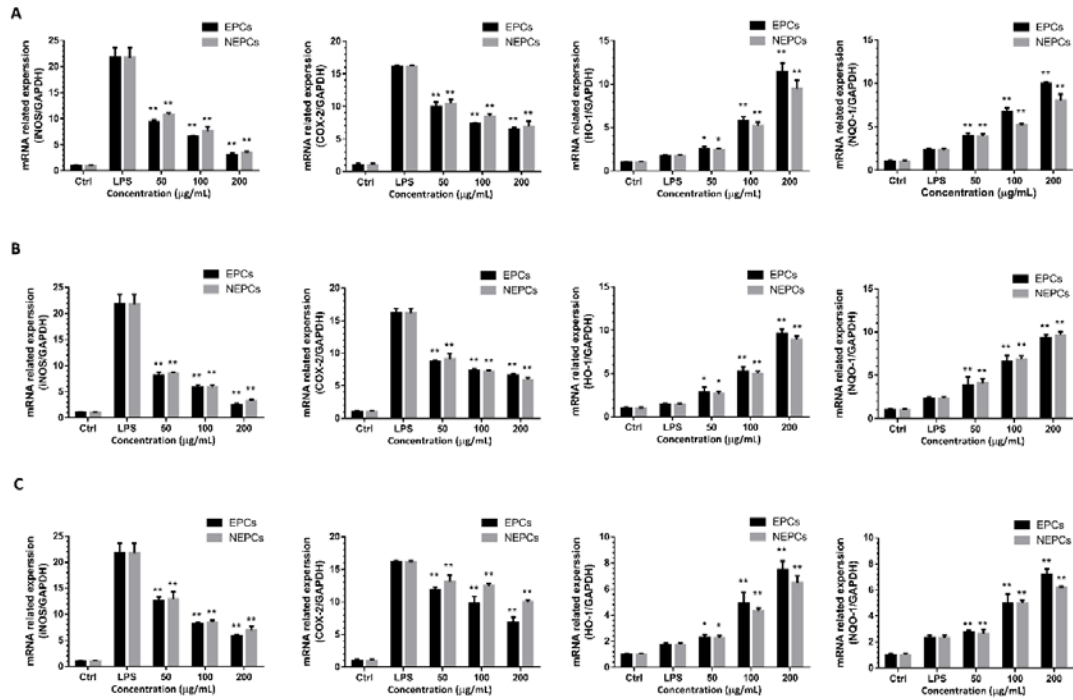


Figure.5. Effects of EPCs and NEPCs from *L. japonica* (A), *U. lactuca* (B), *P. tenera* (C) on mRNA expression of iNOS, COX-2, HO-1 and NQO-1 in activated macrophages. Results were expressed as mean \pm SD (n = 3), * indicates p < 0.05 and ** indicates p < 0.01, when compared to the LPS group.

2.3.6 EPCs and NEPCs elevated the expression levels of antioxidant enzymes in activated macrophages

Elevated expression of HO-1 and NQO-1, two of antioxidant enzymes, have been reported to reduce the overproduction of inflammatory enzymes and proinflammatory cytokines [201]. As shown in Figure.5, EPCs and NEPCs from *L. japonica*, *U. lactuca* and *P. tenera* significantly elevated the mRNA expression level of HO-1 and NQO-1, when compared with LPS group. Specifically, EPCs from *L. japonica*, *U. lactuca* and *P. tenera*, at 200 μ g/mL, potently elevated the mRNA expression of HO-1 by 6.59-, 5.50- and 5.59-fold, respectively. NEPCs from *L. japonica*, *U. lactuca* and *P. tenera*, enhanced the HO-1 mRNA

expression by 5.45-, 5.02- and 4.43-fold, respectively (Figure.5A, 5B & 5C). Similar patterns were obtained in the mRNA expression of NQO-1 (Figure.5A, 5B & 5C). In addition to the mRNA expression, EPCs and NEPCs from three edible seaweeds also greatly upregulated the HO-1 protein expression and their results consistent with the qRT-PCR results (Figure.6A, 6B & 6C).

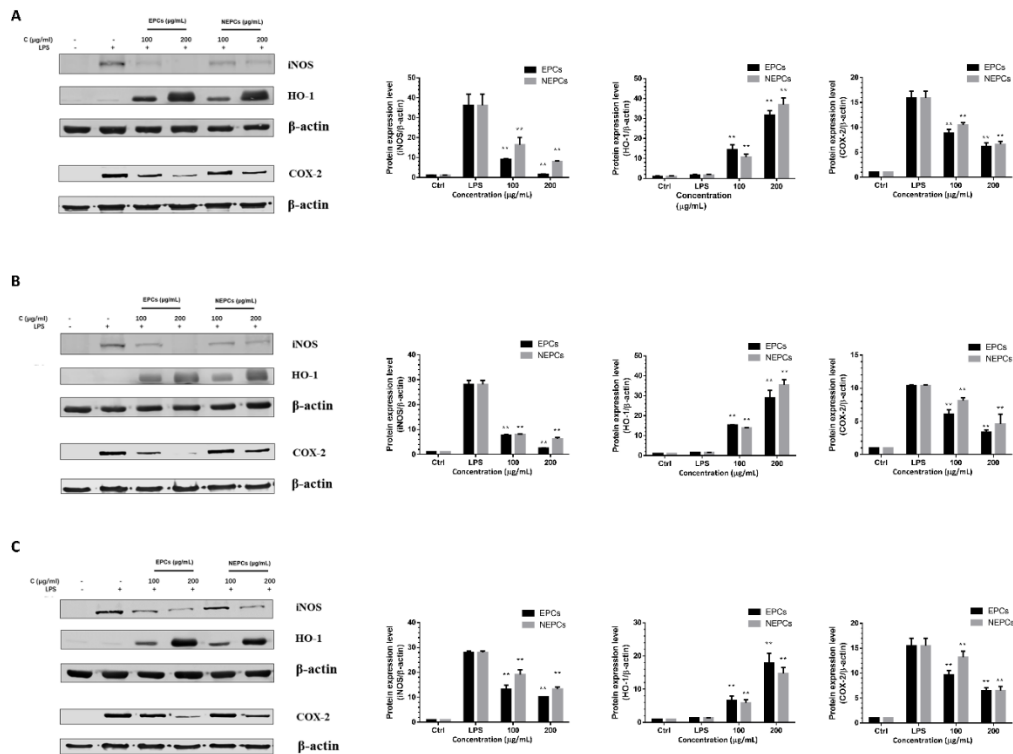


Figure.6. Effects of EPCs and NEPCs from *L. japonica* (A), *U. lactuca* (B), *P. tenera* (C) on protein expression of iNOS, COX-2 and HO-1 in activated macrophages. Results were expressed as mean \pm SD (n = 3). * indicates p < 0.05 and ** indicates p < 0.01 when compared to the LPS group.

2.3.7 EPCs and NEPCs suppressed the viability of colon cancer cells

Lots of phytochemicals with anti-inflammatory and antioxidant capacities also display protective effects on colon cancer [202]. We found that EPCs and NEPCs from three

edible seaweeds did not cause any suppressive effects on the CCD18-Co cells up to 400 µg/mL for 72 hours (Figure. 7A, 7B & 7C). Thus, these concentrations were used to evaluate the anti-colon cancer effects in HCT116 cells. Furthermore, we found that EPCs and NEPCs from three edible seaweeds greatly lowered the cell viability of HCT116 cells in a time- and dose-dependent manner. Specifically, the IC₅₀ values of EPCs from *L. japonica*, *U. lactuca* and *P. tenera* after 48 h treatment were 124.2, 129.5 and 127.2 µg/mL, respectively. The IC₅₀ values of NEPCs from *L. japonica*, *U. lactuca* and *P. tenera* after 48 h treatment were 160.4, 130.5 and 127.5 µg/mL, respectively (Figure.7D, 7E & 7F). Moreover, the IC₅₀ values of EPCs from *L. japonica*, *U. lactuca* and *P. tenera* after 72 h treatment were 105.2, 115.6 and 104.9 µg/mL, respectively. And the IC₅₀ values of NEPCs from *L. japonica*, *U. lactuca* and *P. tenera* after 72 h treatment were 139.3, 95.7 and 94.9 µg/mL, respectively (Figure.7G, 7H & 7I). Our results indicated that EPCs and NEPCs potently suppressed the viability of colon cancer cells, while normal colon cells were not affected at much higher concentrations.

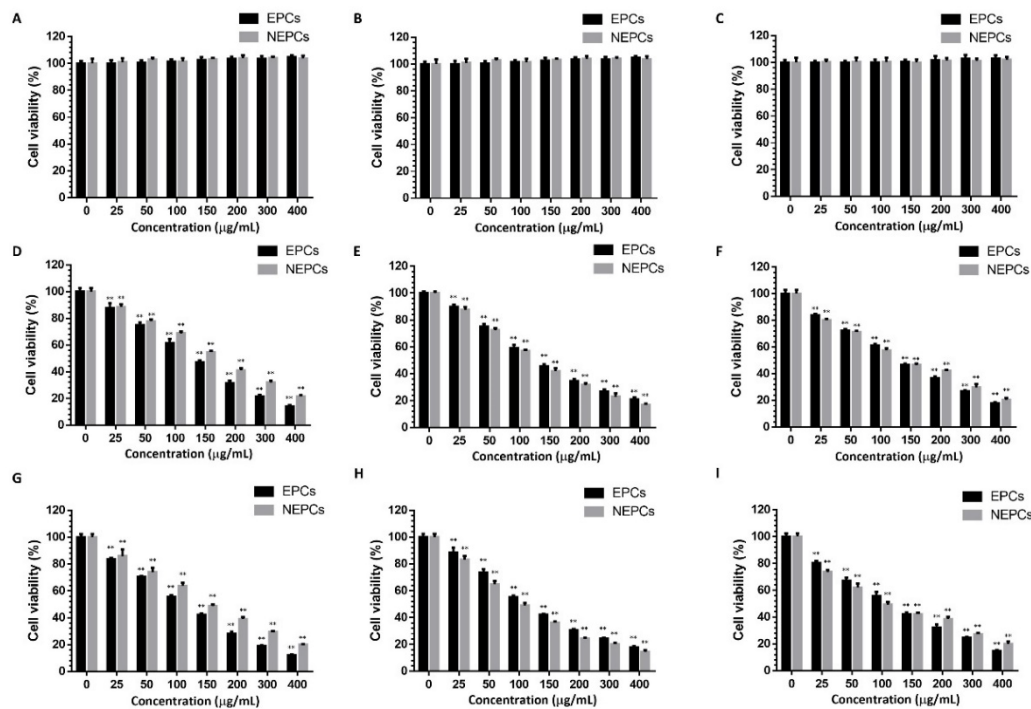


Figure.7. Effects of the EPCs and NEPCs from *L. japonica* (A), *P. tenera* (B) and *U. lactuca* (C) on the growth of CCD18-Co cells for 72 hours; Suppressive effects of the EPCs and NEPCs from *L. japonica* (D), *P. tenera* (E) and *U. lactuca* (F) on HCT116 cells for 48 hours; Suppressive effects of the EPCs and NEPCs from *L. japonica* (D), *P. tenera* (E) and *U. lactuca* (F) on HCT116 cells for 72 hours. Results were presented as mean \pm SD (n = 6). ** indicated $p < 0.01$ in comparison with the untreated control group.

2.3.8 EPCs and NEPCs led cell cycle arrest and apoptosis

Cell proliferation and apoptosis are two important therapeutic targets for cancer [203]. Herein, we selected EPCs and NEPCs at the dose of 150 $\mu\text{g/mL}$ for flow cytometry analysis. EPCs and NEPCs from *L. japonica* and *U. lactuca* noticeably elevated the cell accumulation in the G0/G1 phase. EPCs and NEPCs from *L. japonica* elevated the populations of HCT116 cells in the G0/G1 phase by 1.93- and 1.68-fold, respectively (Figure.8A). Similar patterns were acquired in the analysis of the effects of EPCs and NEPCs from *U. lactuca* on the cell cycle distribution (Figure.8C). Moreover, EPCs from *P. tenera* enhanced the populations of HCT116 cells in the G0/G1 phase by 31.14%, and NEPCs

from *P. tenera* elevated the populations of HCT116 cells in the G2/S phase by 23.50% (Figure.8E). For cell apoptosis analysis, EPCs and NEPCs greatly enhanced apoptotic cell population. Specifically, EPCs from *L. japonica* raised cell population in the early and late apoptosis by 7.76- and 7.25-fold, respectively. NEPCs from *L. japonica*, increased cell population in the early and late apoptosis by 3.01- and 3.80-fold, respectively (Figure.8B). Lastly, the patterns of EPCs and NEPCs from *U. lactuca* and *P. tenera* were consistent with those of EPCs and NEPCs from *L. japonica* (Figure.8D & 8F). These findings indicated that EPCs and NEPCs from three edible seaweeds inhibited the growth of human colon cancer cells via the activation of cell cycle arrest and cellular apoptosis.

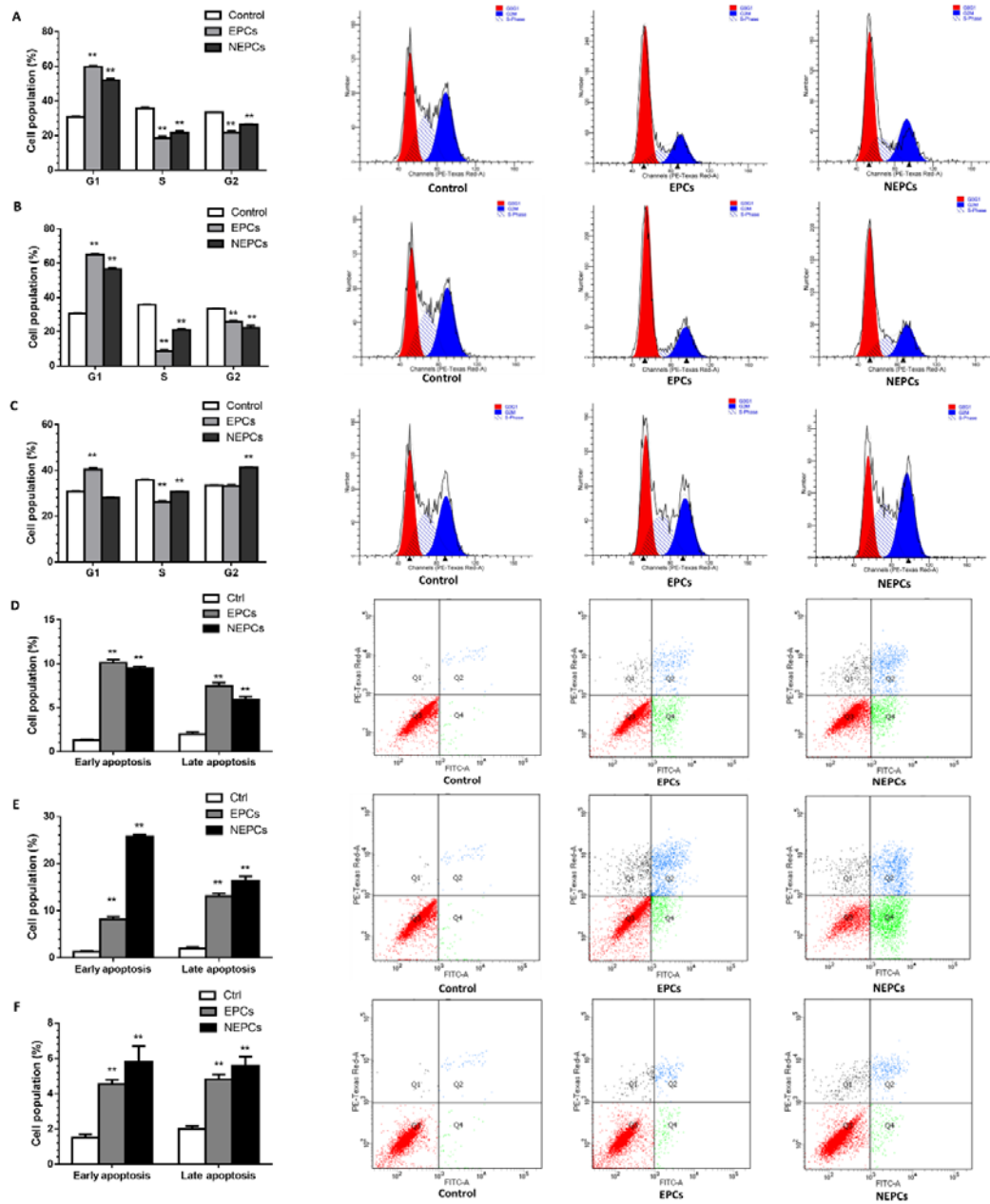


Figure.8. Quantification of cell cycle distribution posed to EPCs and NEPCs from *L. japonica* (A), *U. lactuca* (B), and *P. tenera* (C) and their representative DNA histograms of cell cycle treatment. Quantification of early and late apoptosis posed to EPCs and NEPCs from *L. japonica* (D), *U. lactuca* (E), *P. tenera* (F) and their representative Annexin V/PI co-stain dot plots of cell apoptosis treatment. ** presents $p < 0.01$ in comparison with the untreated control group.

CHAPTER 3

DIETARY INTAKE OF *LAMINARIA JAPONICA* ATTENUATED DEXTRAN SULFATE SODIUM-INDUCED ACUTE COLITIS IN MICE

3.1. Introduction

Inflammatory bowel disease (IBD) is a chronic immune-mediated gastrointestinal disorder with an elevated morbidity rate and characterized by the sign of rectal bleeding, diarrhea and abdominal pain [1]. As an increasing health issue, the pathogenesis of IBD has not been completely understood. Currently, the accepted hypothesis suggests that IBD is a result of an aberrant immune response caused by the complex interaction among the host immune system, gut microbiota and environmental factors [9]. The human gastrointestinal tract harbors more than trillions of microorganisms which have numerous impacts on host's health. The role of gut microbiota in the disease progression of IBD has been highlighted in recent years. The profiles of gut microbiota-derived metabolites, especially for the bile acids (BAs) and short-chain fatty acids (SCFAs), are different between healthy individuals and IBD patients [36, 204]. Dietary pattern, as one of the environmental factors, plays a vital role in promoting colon health by modifying gut microbiota structure and producing bioactive metabolites, thereby controlling the development of IBD [14, 15]. For instance, supplementation of plant-based foods was found to reduce the risk of IBD via alleviating the dysbiosis of gut microbiota in animal studies [16, 29, 30].

Edible seaweeds were used as foodstuff for human nutrition several centuries ago in Pacific and Asian countries. *Laminaria japonica* (LJ), a popular edible brown seaweed, is rich in polysaccharides, minerals, vitamins and phytochemicals [205]. Their biological

properties have been extensively studied in various cell and animal studies. Accurately, LJ and their extracts have been reported to have antioxidant [206], anti-diabetic [207], anti-cancer [208], anti-inflammatory [209], and prebiotic [118] properties in numerous cell studies. Moreover, LJ extracts, especially for polysaccharides, have been reported against chronic metabolic disease via normalizing the gut microbiota [210-213]. Specifically, aqueous extracts from LJ alone or combined with lactic acid bacteria (including *Bifidobacterium*, *Lactobacillus*, and *Streptococcus*) against colitis in DSS-treated mice via repairing colon damage and restoring immune homeostasis [25]. In addition, the whole LJ contains dietary fiber, polysaccharides and polyphenols, which may pose more substantial inhibitory effects against colonic inflammation than isolated components. However, the interaction of the consumption of whole LJ and gut microbiota, as well as the potential anti-inflammatory properties of whole LJ against IBD have not been thoroughly tested. Therefore, the current study was aimed to determine the anti-inflammatory properties of whole LJ against colonic inflammation, as well as their potential mechanism involved.

3.2. Materials and methods

3.2.1 Materials

L. japonica was obtained from Wonderful LLC (Fuzhou, Fujian, China) in January 2020, and was stored at -20°C before use. SCFAs standards, containing propionic acid, butyric acid, acetic acid, valeric acid, isobutyric acid and isovaleric acid, were purchased from Sigma (Sigma-Aldrich, St. Louis, MO, U.S.A.). BAs standards, containing alpha-muricholic acid (α -MCA), beta-muricholic acid (β -MCA), cholic acid (CA), chenodeoxycholic acid (CDCA), tauro-alpha-muricholic acid (T- α -MCA), tauro-beta-muricholic acid (T- β -MCA), taurocholic acid (TCA), tauro-chenodeoxycholic acid (TCDCA), tauro-

omega-muricholic acid (T- ω -MCA), omega-muricholic acid (ω -MCA), murideoxycholic acid (MDCA), deoxycholic acid (DCA), lithocholic acid (LCA), tauro-lithocholic acid (TLCA), ursodeoxycholic acid (UDCA), tauroursodeoxycholic acid (TUDCA) and Taurodeoxycholic acid (TDCA), were purchased from Caymanchem (Cayman Chemical, Ann Arbor, MI, USA).

3.2.2 Animals and experimental design

All animal use was conducted according to the Guidelines from the Institutional Animal Care and Use Committee of the University of Massachusetts Amherst. Sixty male CD-1 (ICR) mice were purchased from Charles River Laboratories (Wilmington, MA). Detailed dietary information was displayed in Figure.9A. Briefly, all mice were randomly divided into six experimental groups (n = 10), containing control group, DSS group, L-LJ group, H-LJ group, DSS-L-LJ group and DSS-H-LJ group, after one week of acclimation. The control group and the DSS group were given an AIN93G diet during the entire experiment. The L-LJ and D-L-LJ group were given an AIN93G diet containing 1% LJ powder. The H-LJ and D-H-LJ were given an AIN93G diet containing 3% LJ powder. The dried LJ powder contains 63.6% dietary fiber, 10.9% crude protein, 1.4% fat, 16.3% ash and 0.24% total carbohydrate. After three weeks, mice in the three colitis groups were given drinking water containing 2.5% (w/v) DSS for one week. All mice were free to access drinking water and diet during the experiment. Mice were monitored basis on food consumption, body weight, blood in stool and fecal consistency throughout the DSS treatment.

3.2.3 Assessment of disease activity index (DAI) and colitis severity

DAI scores were measured based on the blood in fecal (scored as 0-3), stool consistency (scored as 0-3) and body weight loss (scored as 0-3) as in previous studies [66]. Colon tissues were harvested and subjected to hematoxylin and eosin staining as previous study [30]. Histological scores were evaluated based on epithelium (scored as 0-4) and infiltration (scored as 0-4).

3.2.4 Assessment of inflammatory enzymes and cytokines in colon tissues

The colonic mucosa was scraped and homogenized in RIPA buffer (Boston BioProducts, Ashland, MA, USA). Protein samples were obtained by centrifugation at 16,000g for 25 min under 4 °C, and bicinchoninic acid (BCA) assay was conducted to quantify the protein concentrations. Next, the protein was subjected to the quantification of cytokines by U-PLEX ELISA kit (Meso Scale Discovery, Rockville, MD, U.S.A.), according to the manufacturer's procedures. The protein were also assessed to the western blotting analysis according to previous studies [214]. Antibodies of iNOS, COX-2, NF- κ B p65, NF- κ B p50 and β -actin were ordered from Santa Cruz (Dallas, TX, USA).

3.2.5 Quantification of SCFAs

The cecum content was homogenized with 0.05% phosphoric acid and centrifuged at 18 000 g for 20 min. Then, the obtained supernatant was mixed with same volume of ethyl ether, and centrifuged at 18 000 for 15 min. The collected organic phase was assessed by gas chromatography with a flame ionization detector (GC-2010 plus, Shimadzu, Columbia, MD, USA), according to previous studies [215]. Identification of the SCFAs was

based on the retention time of standard compounds, and 4-methylvaleric acid was used as the internal standard.

3.2.6 Quantification of BAs

The freezer-dried feces were combined with a pre-cooled methanol solution. The mixture was ultrasonication for 5 min, followed by centrifugation at 18,000 g for 15 min under 4 °C. The supernatant obtained from the centrifugation was assessed by a single quad LC-MS system (Model 2020, Shimadzu, Kyoto, Japan), at negative electrospray ionization mode. Each standard curve and test sample was injected into a Waters XBridge C18 (100 mm × 4.6 mm, 5 µm) column at a 0.6 mL/min flow rate. The mobile phase consisted of (A) 5 mM ammonium acetate in water and (B) acetonitrile. The elution separation program as given: 0.01-55 min: 25-50% B; 30-36 min: 50-90% B; 36-44 min: 90% B; 40.01-54 min: 90-25% B. The injection volume was 2 µL. The Mass-spectra conditions were optimized according to the previous study [216]. Quantification of BAs based on the retention time of standard compounds, LCA-d4 and TCDCa-d4 were used as internal standards.

3.2.7 Analysis of fecal microbiota

Mice fecal microbiota DNA was isolated by the DNeasy PowerSoil Pro kit (Qiagen), following the manufacturer's instructions. DNA concentration was quantified by a UV-vis spectrophotometer (NanoDrop 2000, Waltham, MA). DNA samples were then assessed to the 16S rRNA sequencing according to the previous study with minor modifications [31]. Briefly, the 16S rRNA genes in the V3-V4 regions were amplified with bar-coded primers (341 F and 806 R). The PCR products were subjected to purification, quantification and pooled into the same equimolar concentration. Sequencing was performed

on Illumina MiSeq platform (Illumina, Inc., San Diego, CA, U.S.A.), after combining the amplicon library.

3.2.8 Statistically analysis

Microbial data were processed using QIIME II software. Statistical significance between groups was evaluated via one-way analysis of variance (ANOVA), followed by Turkey's multiple comparison test or one-way nonparametric ANOVA Kruskal–Wallis's test. A value of $P < 0.05$ was significant.

3.3. Results and discussion

3.3.1 Supplementation of LJ inhibited the symptoms of colitis

The pathological mechanisms behind IBD, a modern refractory disease, is still remain unknown [217]. Edible plant-based food, such as mushrooms and berries, has been shown to exert protective effects against IBD in various experiments colitis model [29, 73]. Nevertheless, only a few limited research focused on the anti-inflammatory properties of edible seaweeds and their bioactive components on intestinal inflammation. The dose of LJ in the current studies were 1% and 3% in the mouse diet, equivalent to around 3.6 g and 10.8 g dried powder of LJ to per person per day [218]. There were no apparent differences in the behavior, body weight, water and food consumption among CTL, L-LJ and H-LJ groups, during the entire experiment. These results suggested that supplementation of whole LJ had no apparent adverse effects on mice. In our study, mice had obviously watery diarrhea and blood in feces after seven days of DSS treatment. DSS stimulation resulted in significant body weight loss, bloody and watery diarrhea. However, LJ supplementation slightly slowed the body weight loss in colitis mice during in the DSS period, especially

for the mice fed with 3% LJ (Figure.9B). Moreover, the severity of symptoms indicated by DAI score was mitigated by the supplementation of LJ in colitis mice (Figure. 9C). In line with the reduced body weight loss and lowered the DAI score, LJ supplementation also prevented colon length shortening and spleen weight enlargement in colitis mice, when compared to the DSS group (Figure.9D & 9E).

Additionally, normal pathological morphology of the colon was observed in the control group. Severe colonic injuries were observed in the DSS group, indicated by the alteration of crypts structure, disappearance of goblet cells and infiltration of immune cells (Figure. 10A & 10B). LJ supplementation partially alleviated colitis, indicated by the less immune cell infiltration, elevation of the goblet cells and crypts, repair of the epithelium and mucosal injuries (Figure. 10A & 10B). Overall, these findings suggested that supplementation of LJ effectively inhibited the severity of colitis and against colonic injuries in colitis mice.

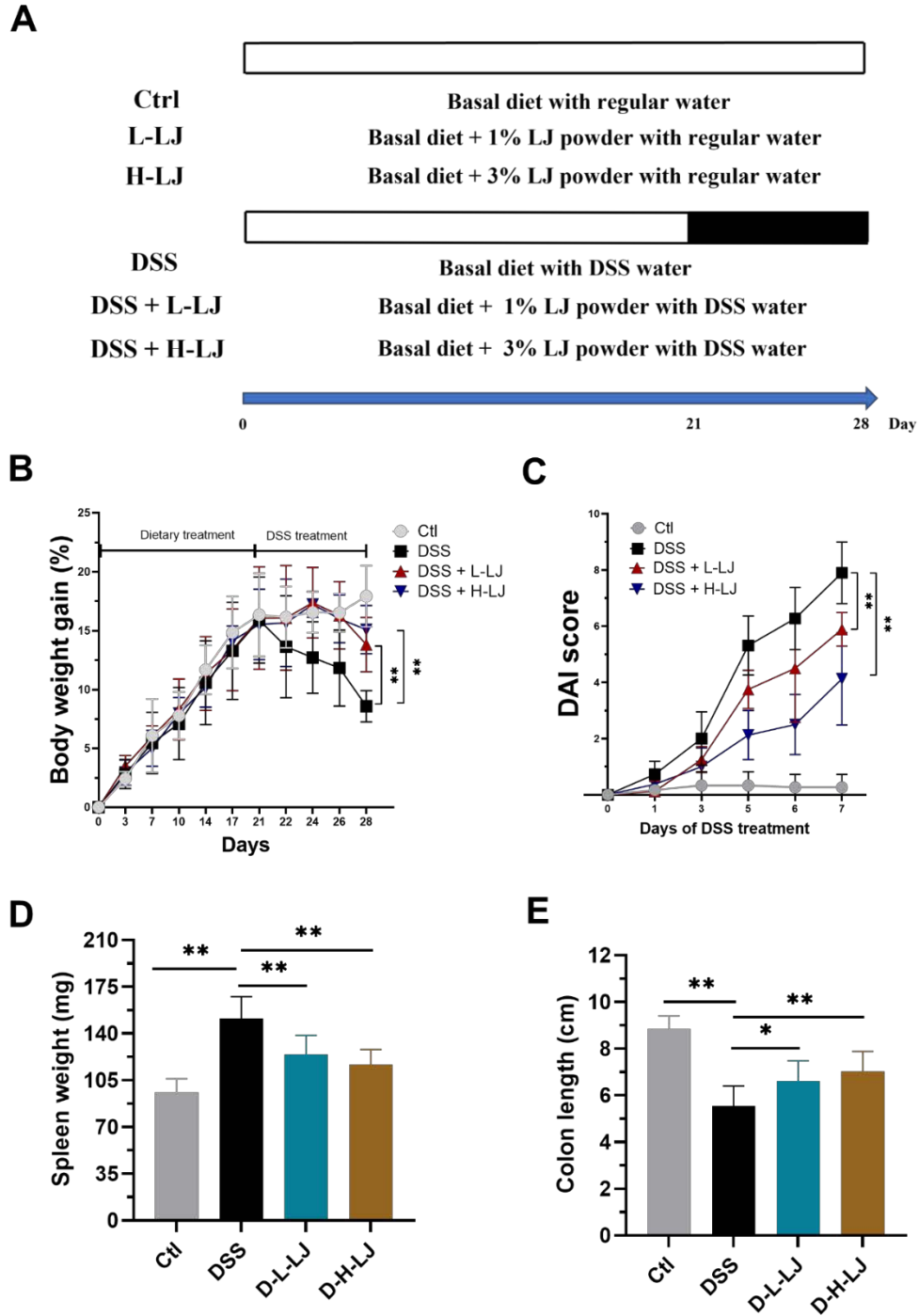


Figure.9. Supplementation of LJ improves the symptoms of DSS-induced acute colitis. (A) Experimental design; (B) the change of body weight (n = 10 per group); (C) DAI score (n = 10 per group); (D) spleen weights (n = 8 - 10 per group); (E) colon length (n = 8 - 10 per group); Results were presented as mean \pm standard deviation (SD). * indicate $p < 0.05$, ** indicate $P < 0.01$.

3.3.2 Supplementation of LJ regulated the production of pro-inflammatory cytokines and inflammatory-related proteins

It has been pointed out that the elevation of pro-inflammatory cytokines in colonic mucosa can be served as a hallmark of the severity of IBD [219]. DSS stimulation slightly elevated the production of pro-inflammatory cytokines in colon mucosa, containing TNF- α , IFN- γ , IL-1 β , IL-2 and IL-6. However, these raised cytokines were dramatically lowered by the supplementation of LJ in colitis mice (Figure.10C). For instance, supplementation of 3% LJ ameliorated the concentrations of TNF- α , IFN- γ and IL-6 by 69.6, 75.4 and 85.6% in colitis, respectively, which did not show the statistical difference compared to the healthy mice.

The activation of nuclear factor- κ B (NF- κ B) can regulate various genes, including proinflammatory cytokines and inflammatory-related enzymes [220]. In the current study, the levels of inflammatory mediators, namely iNOS and COX-2, were slightly elevated in colitis mice in response to the DSS stimulation. Nevertheless, these increased protein expression levels were lowered by the supplementation of LJ in colitis mice, in comparison to the mice treated with DSS alone. For instance, LJ supplementation (3%) lowered the iNOS and COX-2 protein levels by 67.5 and 62.6%, respectively (Fig. 10D). Moreover, similar patterns were acquired in the protein levels of p65 and p50 (Figure. 10D). Overall, these results suggested that LJ supplementation against colitis via enhancing immune homeostasis.

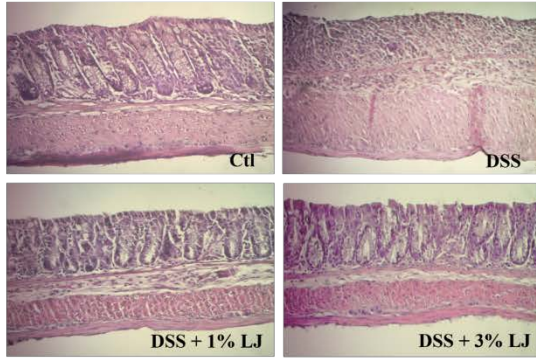
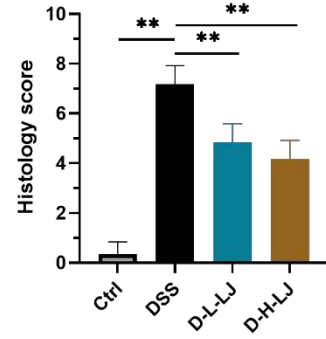
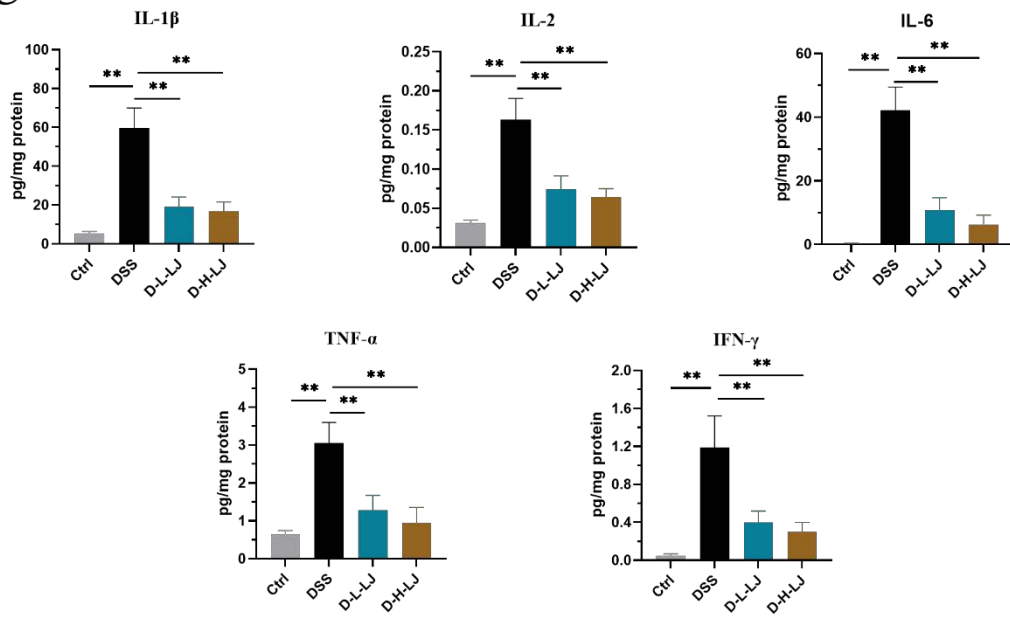
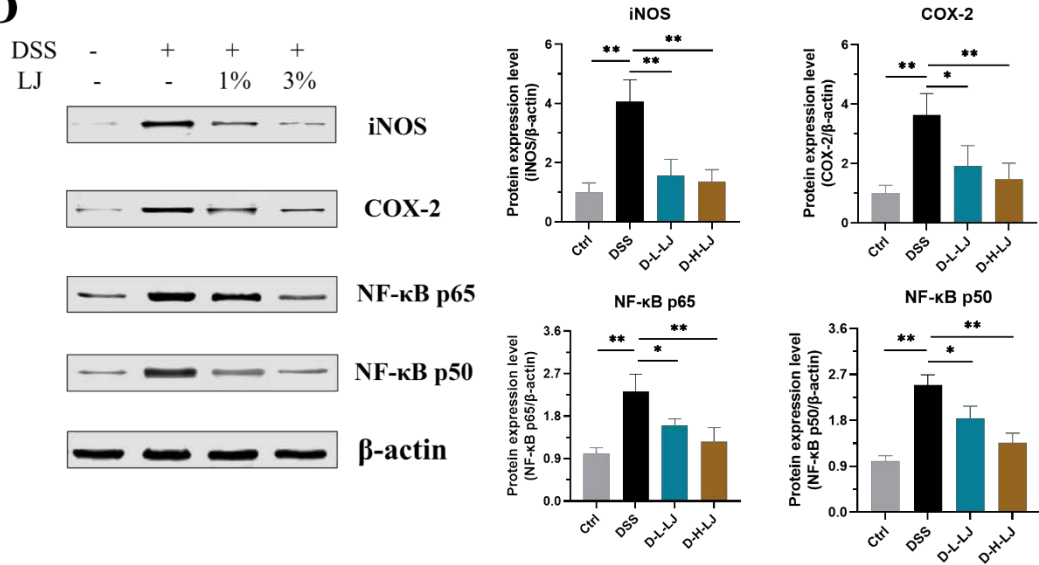
A**B****C****D**

Figure.10. LJ supplementation ameliorated colonic histology damage and regulated the boost of pro-inflammatory cytokines. (A) Representative image of H&E-stained colon (300 × magnification); (B) Histology score of colon damage (n = 6); (C) Quantification of inflammatory cytokines (IL-1 β , IL-2, IL-6, TNF- α , IFN- γ) in colon mucosa (n = 6). (D) Western blotting analysis of inflammatory-related proteins in the colon mucosa (n = 3). Results were presented as mean \pm SD. * indicate $p < 0.05$, ** indicate $P < 0.01$.

3.3.3 Supplementation of LJ ameliorated the production of BAs and SCFAs

BAs and SCFAs are two significant types of gut microbiota-derived metabolites implicated in the pathogenesis of IBD patients [204]. The connections between inflammatory gastrointestinal disorders and bile acids has been known for a long times [221]. In the present study, the levels of 17 BAs, including 8 primary BAs and 9 secondary BAs, in feces were quantified and their profiles varied significantly among the mice from different groups (Table.6). Primary BAs were synthesized in the liver, followed by conjugation with taurine to form conjugated primary BAs. Primary BAs were excreted to gallbladder, and forwarded to the intestinal, where most conjugated BAs go through deconjugation to form unconjugated BAs, and then turn into secondary BAs in the colon through a series of reactions. Gut microbiota is vital in the biotransformation of BAs, thus, dysregulation of BAs cooperated with gut microbiota dysbiosis to induce intestinal inflammation [222, 223]. Herein, the total primary BAs were found to elevation in the DSS group, and the total secondary BAs were found to decrease in the DSS group. Supplementation of LJ shifted the total primary BAs and secondary BAs forward to the control group in colitis mice (Table.1). The levels of most primary BAs (CA, T-CA, T- α -MCA and T- β -MCA) were slightly elevated in colitis in response to the DSS stimulation. Moreover, the levels of secondary BAs (MDCA, DCA, LCA, T-DCA and T-LCA) and two unconjugated primary BAs (α -MCA and β -MCA) were reduced in the DSS group. Additionally, supplementation

of LJ was partially shifted these dysregulated BAs levels forwarded to the control group (Figure.11A & 11B).

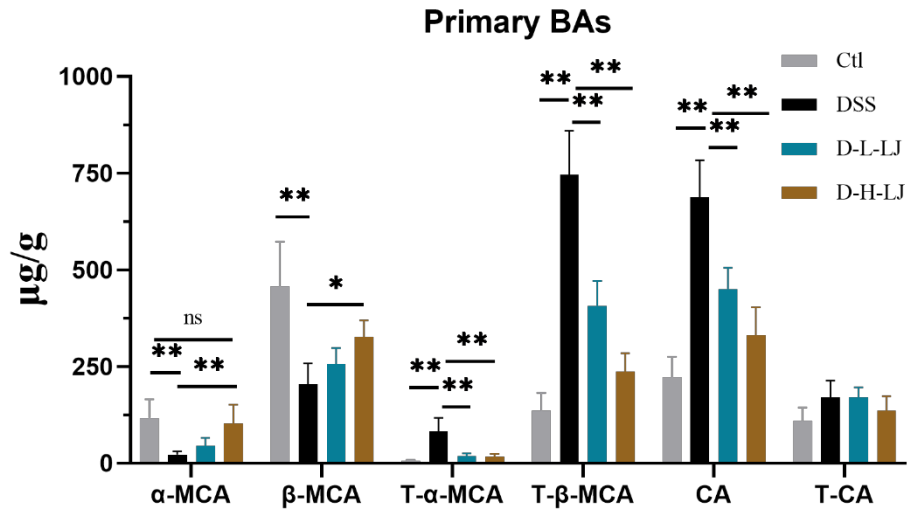
Moreover, SCFAs are the mainly products of indigestible carbohydrates and dietary fiber in the colon [224]. Accumulated studies have targeted the biological properties of SCFAs, especially for their anti-inflammatory capacities [225-227]. Elevations of the SCFAs, particularly for the butyric acid, have been demonstrated to enhance the integrity of gut barrier and alter the inflammatory-related signaling pathway [228, 229]. In the current study, the concentrations of SCFAs in cecum from different groups were quantified (Table.6). Herein, the concentrations of propionic acid, acetic acid, butyric acid and valeric acid were reduced by 69.52, 69.43, 68.38, and 64.51% in colitis mice, respectively, in response to the DSS stimulation (Figure.11C). Moreover, supplementation of LJ shifted the concentrations of butyric acid, acetic acid, propionic acid and valeric acid forward to the control group in colitis mice. Notably, supplementation of 3% LJ in colitis mice shifted the concentrations of butyric acid and propionic acid to similar levels of that in the control group (Figure.11C). Additionally, the total concentrations of SCFAs were declined in the DSS group, which was shifted forward to the control group in colitis mice by supplementation of LJ in a dose-dependent manner (Table.6).

Table.6. BAs and SCFAs content in different groups administrated by LJ.

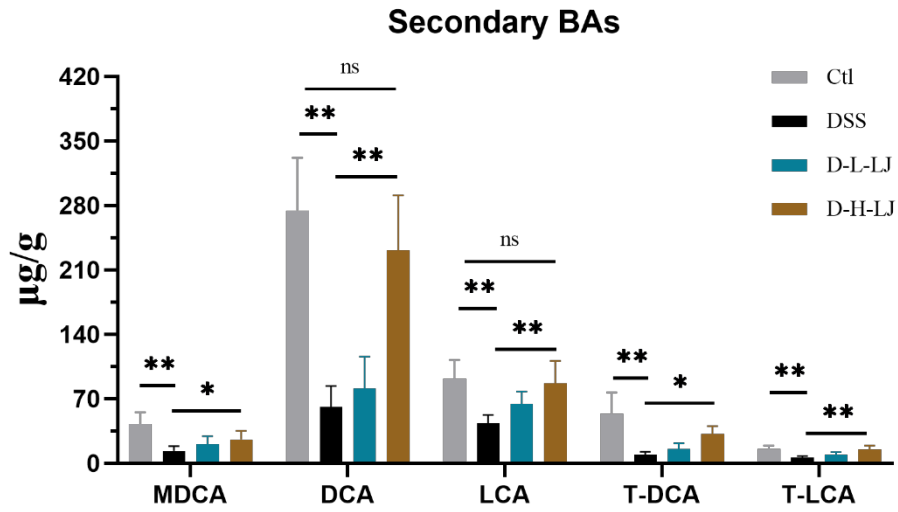
BAs or SCFAs ($\mu\text{g/g}$)	Ctl	L-LJ	H-LJ	DSS	D-L-LJ	D-H-LJ
α -MCA	117.82 \pm 47.75**	113.43 \pm 46.64**	109.61 \pm 47.27**	22.49 \pm 8.90	45.74 \pm 20.21	103.07 \pm 42.90**
β -MCA	457.32 \pm 115.66**	453.02 \pm 127.31**	450.09 \pm 132.52**	204.02 \pm 54.33	256.60 \pm 41.85	326.44 \pm 43.29*
CA	222.47 \pm 53.08**	231.22 \pm 49.18**	229.80 \pm 51.04**	687.62 \pm 95.25	450.85 \pm 54.68**	330.63 \pm 72.80**
T- α -MCA	6.93 \pm 1.64**	7.57 \pm 1.01**	7.31 \pm 1.02**	82.57 \pm 34.78	18.82 \pm 7.23**	16.59 \pm 8.08**
T- β -MCA	136.62 \pm 45.05**	139.74 \pm 50.87**	136.08 \pm 48.74**	745.93 \pm 114.19	407.35 \pm 64.36**	236.56 \pm 48.34**
TCA	109.82 \pm 34.63	115.86 \pm 26.49	112.10 \pm 24.24	170.51 \pm 43.43	169.37 \pm 27.21	136.80 \pm 36.77
CDCA	6.89 \pm 2.55*	6.58 \pm 2.10	6.04 \pm 2.16	2.98 \pm 1.72	3.70 \pm 1.57	4.47 \pm 1.51
TCDCa	5.05 \pm 1.24	4.75 \pm 0.99	4.32 \pm 1.15	2.66 \pm 0.92	4.33 \pm 1.17	4.19 \pm 1.72
Total primary BAs	1062.96 \pm 161.39**	1072.42 \pm 171.24**	1055.39 \pm 175.98**	1918.85 \pm 144.13	1356.80 \pm 58.10**	1158.77 \pm 113.91**
T- ω -MCA	38.26 \pm 8.25*	44.25 \pm 8.45*	43.33 \pm 8.71*	73.20 \pm 17.57	110.74 \pm 11.41*	63.74 \pm 18.59
ω -MCA	411.23 \pm 23.65	431.64 \pm 22.57	429.47 \pm 25.83	491.51 \pm 67.52	494.99 \pm 45.53	534.73 \pm 59.22
MDCA	42.23 \pm 13.23**	40.72 \pm 13.08**	38.13 \pm 9.75**	13.55 \pm 5.36	20.53 \pm 9.06	25.83 \pm 9.65*
UDCA	12.18 \pm 5.26	11.57 \pm 5.03	10.83 \pm 2.95	6.29 \pm 1.83	8.44 \pm 2.30	10.61 \pm 3.32
DCA	274.75 \pm 57.15**	265.52 \pm 64.47**	264.80 \pm 41.97**	61.62 \pm 22.27	81.02 \pm 34.89	231.06 \pm 59.95**
LCA	92.05 \pm 20.52**	88.94 \pm 18.68**	86.18 \pm 23.71**	43.81 \pm 8.64	64.44 \pm 13.67	86.55 \pm 24.81**
T-UDCA	31.94 \pm 14.08	30.49 \pm 13.72	27.70 \pm 12.24	14.97 \pm 7.14	19.21 \pm 6.69	23.45 \pm 6.35
T-DCA	53.69 \pm 23.22**	52.15 \pm 24.45**	51.40 \pm 25.97**	9.22 \pm 3.33	15.85 \pm 5.88	32.07 \pm 8.26*
T-LCA	15.48 \pm 3.87**	14.03 \pm 4.61*	14.31 \pm 3.88*	6.37 \pm 1.91	9.27 \pm 3.16	14.92 \pm 4.51*
Total secondary BAs	972.25 \pm 70.76**	979.36 \pm 63.12**	966.17 \pm 46.45**	720.58 \pm 80.48	824.52 \pm 54.99	1023.01 \pm 80.87**
Acetic acid	1745.71 \pm 313.63**	1831.53 \pm 374.87**	1850.22 \pm 313.74**	533.59 \pm 174.55	1097.10 \pm 228.47*	1412.45 \pm 341.54**
Propionic acid	822.37 \pm 298.82**	723.63 \pm 137.07**	757.46 \pm 117.50**	250.61 \pm 87.47	556.14 \pm 175.83	684.59 \pm 180.53**
Butyric acid	819.29 \pm 280.50**	691.12 \pm 213.87**	667.66 \pm 127.53**	259.05 \pm 98.35	490.98 \pm 102.38	656.02 \pm 142.30**
Iso-butyric acid	31.47 \pm 12.50	31.85 \pm 6.55	26.02 \pm 5.64	23.18 \pm 7.24	29.27 \pm 3.39	36.19 \pm 8.38
Valeric acid	212.04 \pm 39.08**	188.34 \pm 18.20**	187.04 \pm 27.04**	71.70 \pm 17.97	122.15 \pm 30.27	153.93 \pm 40.88**
Iso-valeric acid	105.85 \pm 15.59	102.07 \pm 11.89	96.07 \pm 13.87	99.33 \pm 15.96	96.77 \pm 18.37	91.79 \pm 16.64
Total SCFAs	3736.75 \pm 691.67**	3568.57 \pm 694.01**	3584.49 \pm 554.65**	1237.49 \pm 377.91	2392.43 \pm 357.35*	3034.98 \pm 647.31**

Results were presented as mean \pm SD; * indicate $P < 0.05$, ** indicate $P < 0.01$, compared to the DSS group.

A



B



C

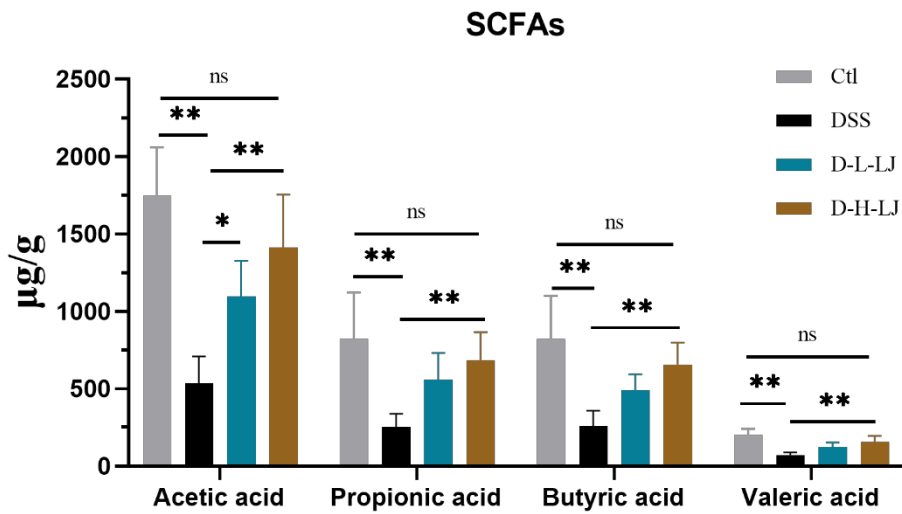


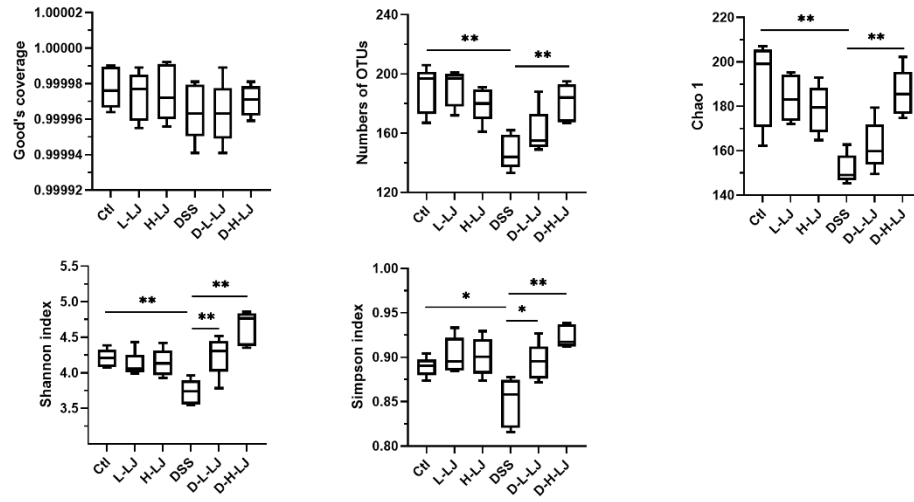
Figure.11. (A) Concentrations of primary BAs in mice feces (n = 5); (B) Concentrations of Secondary BAs in mice feces (n = 5); (C) Concentrations of SCFAs in mice cecal contents (n = 5). Results were presented as mean \pm SD. * indicate $p < 0.05$, ** indicate $P < 0.01$.

3.3.4 Supplementation of LJ reversed the gut microbiota dysbiosis

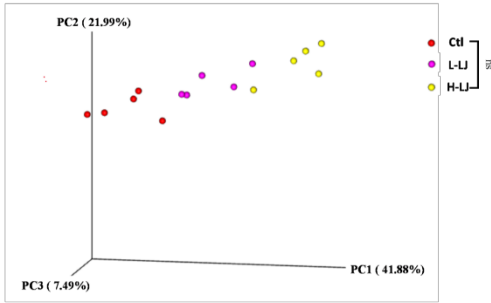
Accumulating evidence indicated that gut microbiota dysbiosis plays a vital role in the pathogenesis and therapeutics of colon colitis [36]. Next, we evaluated the modulatory effects of supplementation of LJ in the gut microbiota diversity. The average value of the coverage index was higher than 0.999 in all groups, indicating the sequencing results were enough to reflect the intestinal flora's actual status in the present study (data not shown). The operational taxonomic units (OTUs) were found to decrease in colitis mice in response to the DSS stimulation. Moreover, supplementation of LJ shifted the OTUs in colitis mice forward to the control group. supplementation of LJ did not change the OTUs in healthy mice. In line with the elevated OTUs, LJ supplementation also elevated the flora diversity indices (Shannon index and Simpson index) and flora abundance indices (Chao 1) in colitis mice (Figure.12A). Besides, the relative similarity of bacteria was visualized by principal coordinate analysis (PCoA) with weighted UniFrac index. As shown in Figure.12B, the three first principal coordinates exhibited a 71.36% variation between all groups. Supplementation of LJ has a trend to shift the gut microbiota forward to the colitis mice, still there was no statistical difference among all healthy groups (Figure.12B). However, LJ supplementation shifted the gut microbiota forward to the control group in colitis mice (Figure.12C). Additionally, we found that the proportion of Proteobacteria, *Enterococcus*, *Escherichia-shigella* and *Parabacteroides* were expanded in the DSS group. On the other hand, Firmicutes, *Lactobacillus*, *Robinsoniella* were presented with a higher abundance in the

control group. Supplementation of LJ shifted this dominant microbiota in both colitis mice and healthy mice (Figure.12D & 12E).

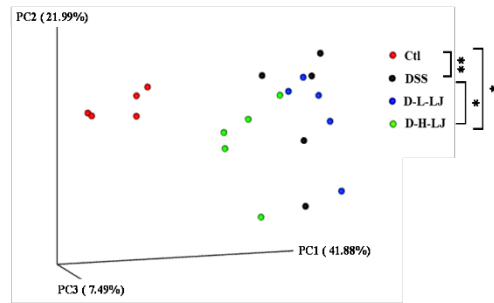
A



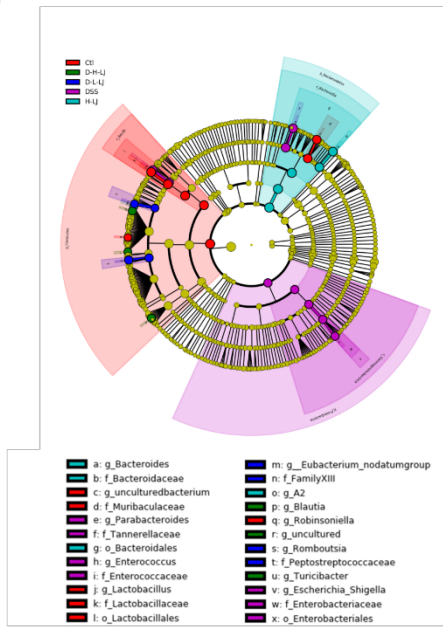
B



C



D



E

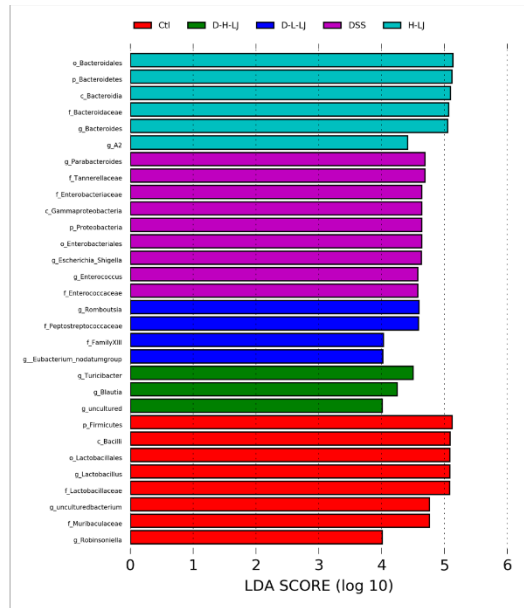


Figure.12. LJ supplementation partially reversed gut microbiota dysbiosis in DSS-treated mice. (A) Modulatory effects of LJ on the α -diversity of gut microbiota, including number of OTUs, Shannon index, Simpson index and Chao 1 index (n = 5); (B) Modulatory effects of LJ on the beta diversity of gut microbiota in healthy mice, presented as principal coordinates analysis (PCoA) plot (n = 5). (C) Modulatory effects of LJ on the beta diversity of gut microbiota in colitis mice, presented as PCoA plot (n = 5). (D) taxonomic cladogram obtained from LEfSe analysis ($\alpha < 0.1$); (E) Distribution of histogram based on the LDA scores (LDA score > 4 and $\alpha < 0.1$); Results were presented as mean \pm SD. * indicate $p < 0.05$, ** indicate $P < 0.01$.

The taxonomic composition at the phylum and genus levels in each group were presented in Table.7. At the phylum levels, the proportions of Actinobacteria, Bacteroidetes, Firmicutes and Proteobacteria were the dominant microbiota. Bacteroidetes are strongly associated with the disease progression of IBD and Firmicutes favor anti-inflammatory properties [230]. In the current study, there was a significant reduction in the abundance of Firmicutes and Actinobacteria, as well as a significant elevation in the abundance of Bacteroidetes and Proteobacteria in the DSS group, in comparison to the control group. Moreover, LJ supplementation shifted the abundance of Actinobacteria, Bacteroidetes, Firmicutes and Proteobacteria in colitis mice forward to the control group. Specifically, the proportion of Firmicutes reduced from 61.27% in the control group to 41.49% in the DSS group (Figure. 13A & 13B). In comparison, the proportion of Bacteroidetes increased from 26.77% in the control group to 44.97% in the DSS group (Figure. 13A & 13B). Supplementation of 3% LJ partially reversed the abundance of Firmicutes from 41.49% to 56.49% and the abundance of Bacteroidetes from 44.97% to 31.70% in colitis mice. Similar patterns were obtained in the abundance of Actinobacteria and proteobacteria, respectively (Figure.13A & 13B).

At the genus levels, the abundance of *Akkermansia*, *Escherichia-Shigella* and *Enterococcus* were elevated by 2.72-, 10.58- and 9.51-fold in the DSS group, respectively, in

comparison to the control group. Moreover, the abundance of *Lactobacillus* and *Blautia* was reduced by 80.5 and 50% in the DSS group, respectively, in comparison to the control group. *Lactobacillus* has shown anti-inflammatory properties in various study and a higher proportion of *Lactobacillus* have been reported to ameliorate colonic inflammation [113, 114]. *Blautia* is vital in the host physiological dysfunction. A lower abundance of *Blautia* was identified in IBD and colon cancer patients [231, 232]. The relative abundance of *Lactobacillus* dramatically dropped from 35.15% in the control group to 6.87% in the DSS group. Supplementation of LJ elevated the abundance of *Lactobacillus* in colitis mice, in comparison to the mice treated with DSS alone. A similar pattern was obtained in the relative abundance of *Blautia* (Figure.13C). A higher proportion of *Akkermansia* has been identified in colitis mice and IBD patients, which is probably due to their ability to degrade the mucin, stimulation of LPS activity [233, 234]. The proportion of *Akkermansia* was elevated by 2.73-fold in colitis mice, in response to DSS stimulation. Supplementation of 3% LJ slightly reduced the abundance of *Akkermansia* by 32% in colitis mice, in comparison to the DSS group (Figure.13C). Moreover, *Escherichia-Shigella* and *enterococcus* have been reported to induce colitis in various mice model [235, 236]. Their relative abundance was found to increase in the colitis mice, and supplementation of LJ dramatically lowered the raised relative abundance of *Escherichia-Shigella* and *enterococcus* in colitis mice (Figure.13C).

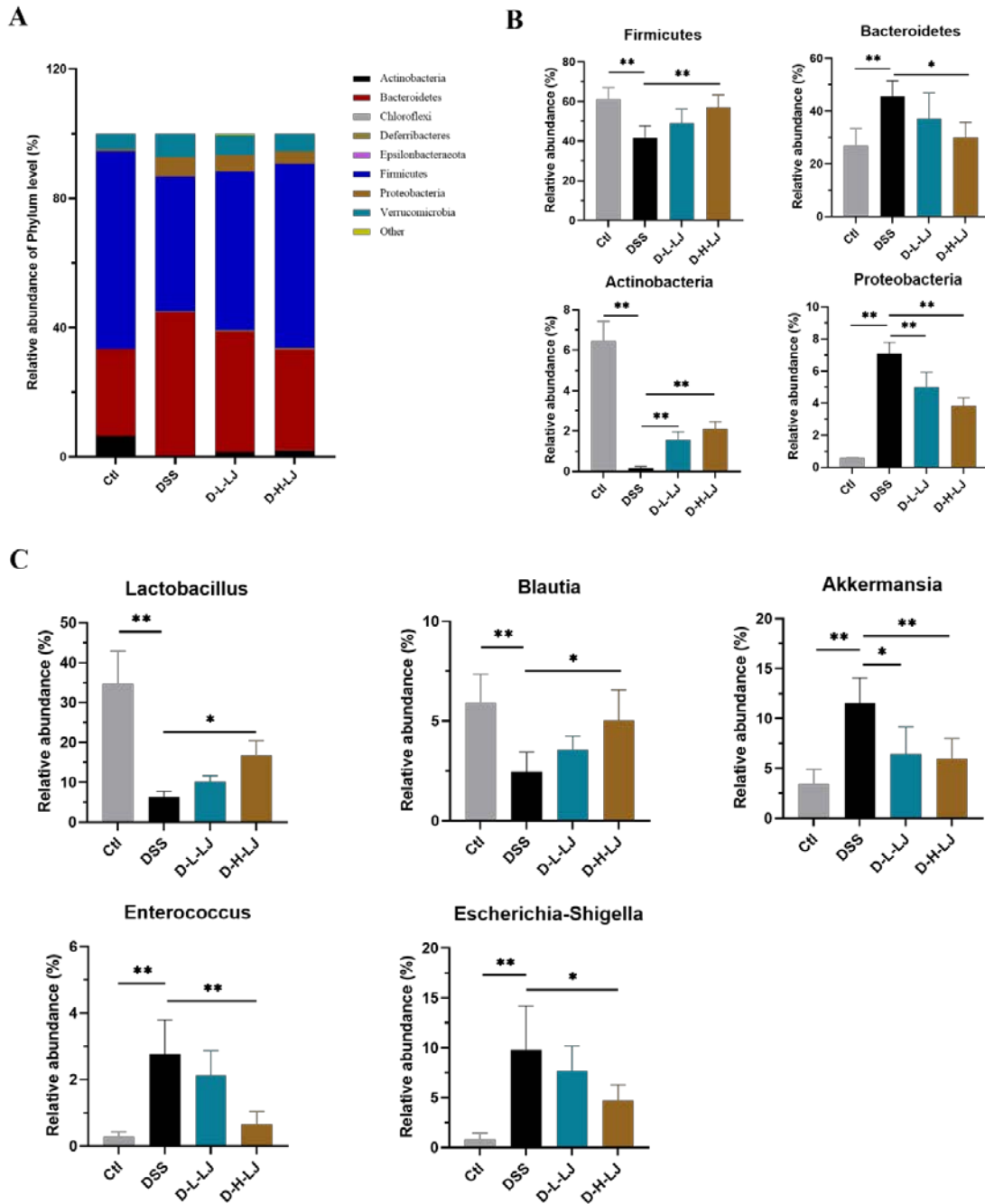


Figure.13. LJ supplementation partially reverses the composition of gut microbiota. (A) Relative abundance of gut microbiota at the Phylum level (n = 5); (B) Relative abundance of Bacteroidetes, Firmicutes, Proteobacteria and Actinobacteria (n = 5); (C) Relative abundance of *Lactobacillus*, *Blautia*, *Akkermansia*, *Escherichia-Shigella* and *Enterococcus* at genus levels. Results were presented as mean \pm SD (n = 5). * indicate $p < 0.05$, ** indicate $P < 0.01$.

Table.7. Relative abundance of gut microbiota with significant differences among different group

Relative abundance (%)	Ctl	L-LJ	H-LJ	DSS	D-L-LJ	D-H-LJ
Actinobacteria	6.44 ± 0.72**	5.61 ± 0.66**	5.09 ± 0.35**	0.16 ± 0.08	1.54 ± 0.28*	2.09 ± 0.12**
Bacteroidetes	26.77 ± 3.49**	25.26 ± 3.62**	29.83 ± 2.53**	44.97 ± 4.82	37.80 ± 3.01	31.70 ± 1.83**
Firmicutes	61.27 ± 3.61**	62.98 ± 2.86**	58.08 ± 1.88**	41.49 ± 4.01	48.61 ± 3.74	56.49 ± 2.73**
Proteobacteria	0.56 ± 0.13**	0.43 ± 0.10**	0.39 ± 0.17**	6.02 ± 1.45	4.97 ± 0.64**	3.82 ± 0.44**
<i>Lactobacillaceae</i>	32.96 ± 6.02**	31.88 ± 6.09**	29.91 ± 4.45**	7.13 ± 2.83	13.10 ± 2.90	19.30 ± 3.76**
<i>Lachnospiraceae</i>	24.29 ± 2.29**	22.94 ± 4.23**	20.65 ± 3.51**	8.85 ± 1.51	14.27 ± 3.62*	23.11 ± 6.32**
<i>Akkermansiaceae</i>	3.96 ± 1.63**	4.00 ± 1.15**	3.41 ± 1.13**	8.49 ± 1.40	6.28 ± 1.10	5.80 ± 1.38*
<i>Enterobacteriaceae</i>	0.87 ± 0.41**	0.58 ± 0.40**	0.27 ± 0.13**	9.14 ± 2.21	7.48 ± 2.20	5.22 ± 2.23*
<i>Enterococcaceae</i>	0.27 ± 0.12**	0.14 ± 0.09**	0.14 ± 0.05**	2.88 ± 0.92	1.59 ± 0.49**	1.37 ± 0.31**
<i>Staphylococcaceae</i>	0.05 ± 0.02**	0.05 ± 0.02**	0.04 ± 0.03**	1.26 ± 0.24	0.48 ± 0.16**	0.15 ± 0.04**
<i>Lactobacillus</i>	34.60 ± 8.26**	31.62 ± 3.17**	31.26 ± 5.60**	5.25 ± 1.39	10.18 ± 1.51	16.72 ± 3.73*
<i>Akkermansia</i>	3.44 ± 1.49**	3.89 ± 1.22**	4.77 ± 1.00**	11.53 ± 2.51	6.44 ± 2.72**	5.97 ± 2.03**
<i>Escherichia-Shigella</i>	0.84 ± 0.63**	0.65 ± 0.42**	0.34 ± 0.16**	9.59 ± 3.51	7.64 ± 2.53	4.62 ± 1.39**
<i>Blautia</i>	6.83 ± 1.24**	5.74 ± 0.99**	5.42 ± 1.30**	2.45 ± 0.98	3.58 ± 0.69	5.33 ± 1.68**
<i>Enterococcus</i>	0.29 ± 0.15**	0.16 ± 0.05**	0.09 ± 0.03**	2.77 ± 1.03	2.13 ± 0.75	0.65 ± 0.39**
<i>Lachnoclostridium</i>	2.43 ± 0.87**	1.97 ± 0.22**	2.23 ± 0.60**	1.01 ± 0.41	1.40 ± 0.66	1.98 ± 0.66*
<i>Staphylococcus</i>	0.63 ± 0.29**	0.81 ± 0.14**	0.74 ± 0.12**	3.05 ± 0.82	1.76 ± 0.70*	1.06 ± 0.63**
<i>Oscillibacter</i>	0.03 ± 0.02**	0.06 ± 0.03**	0.06 ± 0.03**	1.36 ± 0.38	0.67 ± 0.42**	0.39 ± 0.15**
<i>Clostridioides</i>	0.23 ± 0.08**	0.17 ± 0.06**	0.18 ± 0.08**	0.06 ± 0.01	0.05 ± 0.02	0.15 ± 0.05*
<i>Roseburia</i>	0.14 ± 0.06**	0.13 ± 0.03**	0.14 ± 0.04**	0.008 ± 0.004	0.001 ± 0.004	0.09 ± 0.04*

Results were presented as mean ± SD; * indicate P < 0.05, ** indicate P < 0.01, compared to the DSS group.

3.3.5 Correlation analysis between the gut microbiota and colitis indices

According to the findings above, colitis mice were accompanied by the dysregulation of pro-inflammatory cytokines, SCFAs and BAs, as well as dysregulation of the relative abundance of identified gut microbiota. Pearson correlation was conducted to evaluate the relationship between the gut microbiota and colitis parameters (Figure.14). Potential harmful bacteria were positively correlated to the colitis symptoms. For instance, *Enterococcus* was positively associated with the alteration of cytokines, and negatively correlated with the production of SCFAs and secondary BAs. Similar patterns were obtained in the *Staphylococcus*, *Oscillibacter* and *Escherichia-Shigella*. Additionally, the potentially beneficial bacteria, namely *Lactobacillus*, *Blautia* and *Roseburia*, were negatively correlated to the levels of cytokines, and positively correlated with the concentrations of SCFAs and secondary BAs.

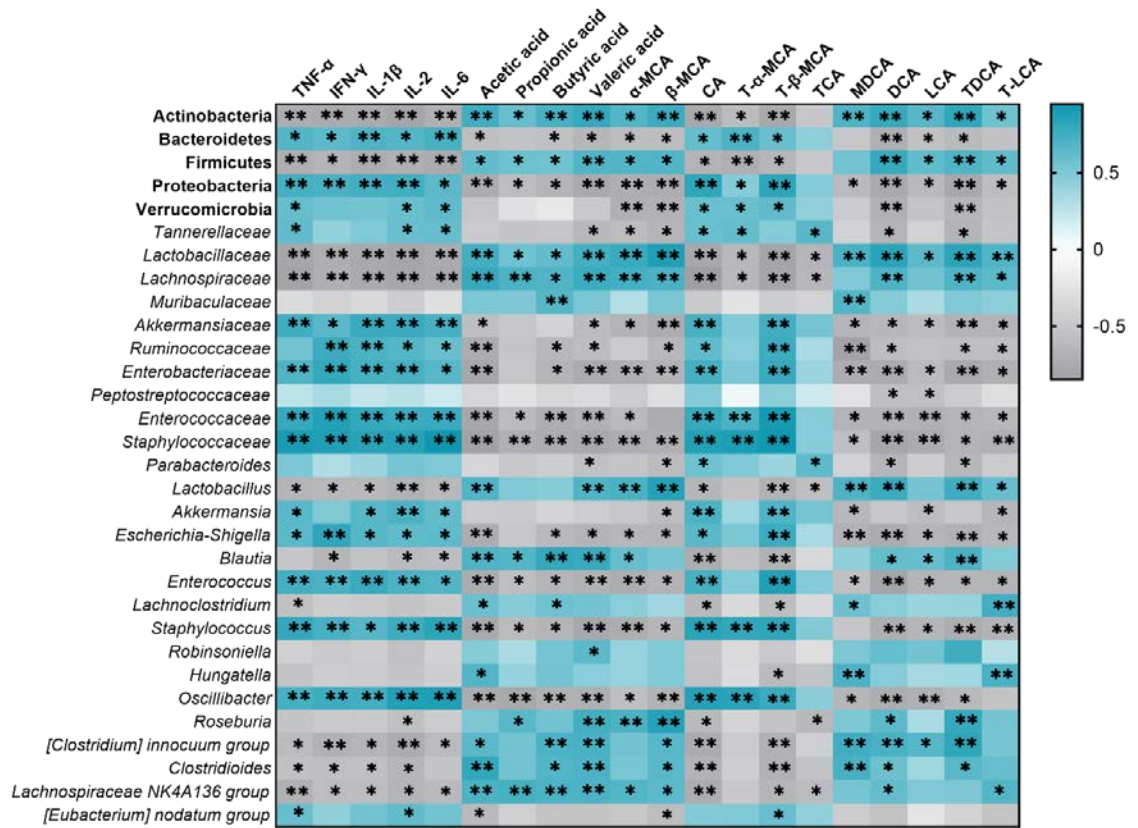


Figure.14. The correlation between the bacterial and cytokines or SCFAs or BAs. Results were presented as mean ± SD. * indicate p < 0.01, ** indicate P < 0.001.

CHAPTER 4

ANTI-INFLAMMATORY PROPERTIES OF PORPHYRA TENERA IN MICE WITH DEXTRAN SODIUM SULFATE-INDUCED COLITIS

4.1. Introduction

The incidence and prevalence of inflammatory bowel disease (IBD), a chronic and relapsing intestinal inflammation represents by ulcerative colitis (UC) and Crohn's disease (CD), has increased rapidly in worldwide in the past few decades [16]. However, the pathogenesis of IBD is unclear. Accumulated studies suggested that IBD is intensely close to unhealthy dietary patterns and an imbalance in gut microbiota [237]. Gut microbiota is vital in regulating immune system homeostasis, host metabolism and pathogen infection [238, 239]. The gut microbiota and their metabolites in IBD patients was differ from healthy individuals [204]. Hence, altering the dysbiosis of gut microbiota can be seen as a novel strategy for preventing and treating IBD. Furthermore, bioactive components from natural resources positively affect human health, including enhancing intestinal function and reducing the risk of metabolic diseases. Dietary intake of plant-based foods was found to have a lower risk of IBD and colon cancer [240].

Edible seaweeds are rich in bioactive components, including dietary fibers, fatty acids, minerals, and polyphenols, which are subjected to accumulated studies focused on their biological properties [22]. *Porphyra tenera* (PT), one of the popular edible seaweeds, has been consumed by Asian countries for a long time. Phenolic compounds isolated from the PT have been reported with antioxidant properties [157, 241]. Bioactive compounds from PT posed antioxidative, anti-inflammatory, anti-colon cancer and immunostimulatory

properties in the cell studies [214, 242, 243]. Moreover, bioactive compounds from PT inhibit ear edema UV irradiation-induced photoaging in the animal studies [244, 245]. While, PT extracts were found to have anti-inflammatory properties in dextran sodium sulfate (DSS)-induced colitis in mice, which was accompanied by modulating gut microbiota composition in colitis mice [80]. Additionally, supplementation of PT extracts were found to enhance the immune functions in healthy adults [246]. These studies have reported that isolated bioactive components from PT pose various beneficial effects. Nevertheless, the role of the whole PT in intestinal inflammation disorders has not been tested yet. Therefore, the current study evaluates the anti-inflammatory effects of PT in DSS-induced colitis in mice, as well as elucidates the prebiotic effects of PT on gut microbiota dysbiosis in colitis mice.

4.2. Materials and Methods

4.2.1 Materials

P. tenera was obtained from PlantGift LLC (Haozhou, Anhui, China) in January of 2020, and was stored at -20°C before use. SCFAs standards, containing propionic acid, butyric acid, acetic acid, valeric acid, isobutyric acid and isovaleric acid, were purchased from Sigma (Sigma-Aldrich, St. Louis, MO, U.S.A.). BAs standards, containing alpha-muricholic acid (α -MCA), beta-muricholic acid (β -MCA), cholic acid (CA), chenodeoxycholic acid (CDCA), tauro-alpha-muricholic acid (T- α -MCA), tauro-beta-muricholic acid (T- β -MCA), taurocholic acid (TCA), tauro-chenodeoxycholic acid (TCDCA), tauro-omega-muricholic acid (T- ω -MCA), omega-muricholic acid (ω -MCA), murideoxycholic acid (MDCA), deoxycholic acid (DCA), lithocholic acid (LCA), tauro-lithocholic acid (TLCA), ursodeoxycholic acid (UDCA), tauroursodeoxycholic acid (TUDCA) and

Taurodeoxycholic acid (TDCA), were acquired from Caymanchem (Cayman Chemical, Ann Arbor, MI, USA).

4.2.2 Animals and experimental design

Sixty male CD-1 (ICR) mice were ordered from the Charles River Laboratories (Wilmington, MA). All animal use was conducted according to the Guidelines from the Institutional Animal Care and Use Committee of the University of Massachusetts Amherst. After one week of acclimation, all mice were randomly divided into three experimental groups (n = 20), receiving a standard diet containing 0, 1 and 3% PT powder and regular water. After three weeks of dietary treatment, mice in each dietary group were randomly divided into two sub-groups, receiving regular drinking water with or without 2.5% DSS water, which resulted in 6 dietary groups: the control group was fed with AIN-93G diet (Dyets Inc., Bethlehem, PA, USA) and normal water; the L-PT group was fed with AIN-93G diet containing 1% PT powder and normal water; the H-PT group was fed with AIN-93G diet containing 3% PT powder and normal water; The DSS-group was treated with AIN-93G diet and 2.5% (w/v) DSS water; DSS-L-PT group was treated with 1% PT supplemented AIN93G diet and 2.5% (w/v) DSS water; DSS-H-PT group was treated with 3% PT supplemented AIN93G diet with 2.5% (w/v) DSS water. The PT dried powder used in the present study contains 42.4% total dietary fiber, 6.1% total carbohydrate, 27.5% crude protein, 2.7% fat and 8.7% ash. All mice were free to access drinking water and diet during the entire experiment. Mice were monitored for food consumption, body weight, blood in stool and fecal consistency throughout the experiments.

4.2.3 Disease activity index (DAI) and colonic histology analysis

DAI scores were determined based on the rectal bleed (scored as 0-3), stool consistency (scored as 0-3) and body weight loss (scored as 0-3). Histological analysis, based on the epithelium (scored as 0-4) and infiltration (scored as 0-4), as previous study [31].

4.2.4 Enzyme-linked immunosorbent and immunoblotting assay

The colon mucosa was scraped and homogenized in RIPA buffer (Boston BioProducts, Ashland, MA, USA). Protein samples were obtained by centrifugation at 16,000g for 25 min under 4 °C. The concentrations of cytokines were determined by a ELISA kits (Meso Scale Discovery, Rockville, MD, USA), followed by the manufacturer's instructions. The proteins were also subjected to the western blotting analysis according to previous studies [214]. Antibodies of iNOS, COX-2, NF- κ B p65, NF- κ B p50 and β -actin were ordered from Santa Cruz (Dallas, TX, USA).

4.2.5 Analysis of gut microbiota

Mice fecal microbiota DNA was isolated by the DNeasy PowerSoil Pro kit (Qiagen), following the protocol of the manufacturer. Samples were then assessed to the 16S rRNA sequencing according to the previous study with minor modifications [31]. Briefly, the 16S rRNA genes in the V3-V4 regions were amplified with barcoded primers (341 F and 806 R). The PCR products were subjected to purification, quantification, and pooled into the same equimolar concentration. Sequencing was performed on Illumina MiSeq platform (Illumina, Inc., San Diego, CA, U.S.A.), after combining the amplicon library.

4.2.6 Determination of short-chain fatty acids (SCFAs)

The cecum content was homogenized with 0.05% phosphoric acid and centrifuged at 18 000 g for 20 min. Then, the obtained supernatant was mixed with same volume of ethyl ether, and centrifuged at 18 000 for 15 min. The collected organic phase was assessed by gas chromatography with a flame ionization detector (GC-2010 plus, Shimadzu, Columbia, MD, USA), according to previous studies [215]. Quantification of the SCFAs was based on the retention time of standard compounds, and 4-methylvaleric acid was used as the internal standard.

4.2.7 Quantification of bile acids (BAs)

The freezer-dried feces samples were combined with a pre-cooled methanol solution containing ISs. The mixture was ultrasonication for 5 min, followed by centrifugation at 18,000 g for 15 min under 4 °C. The supernatant was collected and analyzed by a liquid chromatography-mass spectrometry (LC-MS) system (Model 2020, Shimadzu, Kyoto, Japan). The LC-MS was fitted with a Waters XBridge C18 column (100 mm × 4.6 mm, 5 µm) and nitrogen was used as carrier gas at 15L/min. The mobile phase consisted of (A) 5 mM ammonium acetate in water and (B) acetonitrile. The elution separation program as follows: 0.01-55 min: 25-50% B; 30-36 min: 50-90% B; 36-44 min: 90% B; 40.01-54 min: 90-25% B. The injection volume was 2 µL at a 0.6 mL/min flow rate. Data were acquired in negative ESI mode, using an interface voltage -3500V, the heat block temperature and desolvation line temperature set to 400 and 250°C, respectively. Identification of BAs based on the retention time of standard compounds, LCA-d4 and TCDCA-d4 were used as internal standards.

4.2.8 statistically analysis

Microbial data were assessed by QIIME II software. P value was calculated using one-way analysis of variance (ANOVA), followed by Turkey's multiple comparison test or one-way nonparametric ANOVA Kruskal–Wallis's test. Difference with $P < 0.05$ was significant.

4.3. Results

4.3.1 Intake of PT relieve the symptoms of colitis

No apparent differences in the average body weight, spleen weight, colon length and DAI scores among the control, L-PT and H-PT, indicating the dose of PT in the present study did not cause apparent toxic effects. Moreover, the body weight was declined in the DSS group during the DSS treatment period, the declined body weight was elevated by the intake of PT in colitis mice. (Figure.15A & 15C). The DAI scores in the DSS group were gradually elevated during the DSS treatment period, administration of PT dose-dependent reduced the DAI scores in the colitis mice, in comparison to the DSS group (Figure.15B). Additionally, oral intake of PT reduced the spleen weight and elevated the colon length in the colitis mice, when compared to the mice treated with DSS alone (Figure. 15D & 15E). Furthermore, histological analysis suggested that severe colitis was found in the DSS group, indicated by the loss of goblet cells and epithelial cells, increasing the infiltration of inflammatory cells and disruption of the structure of the crypts. Oral administration of PT dose-dependent alleviated these abnormalities induced by the DSS stimulation (Figure. 15F & 15G).

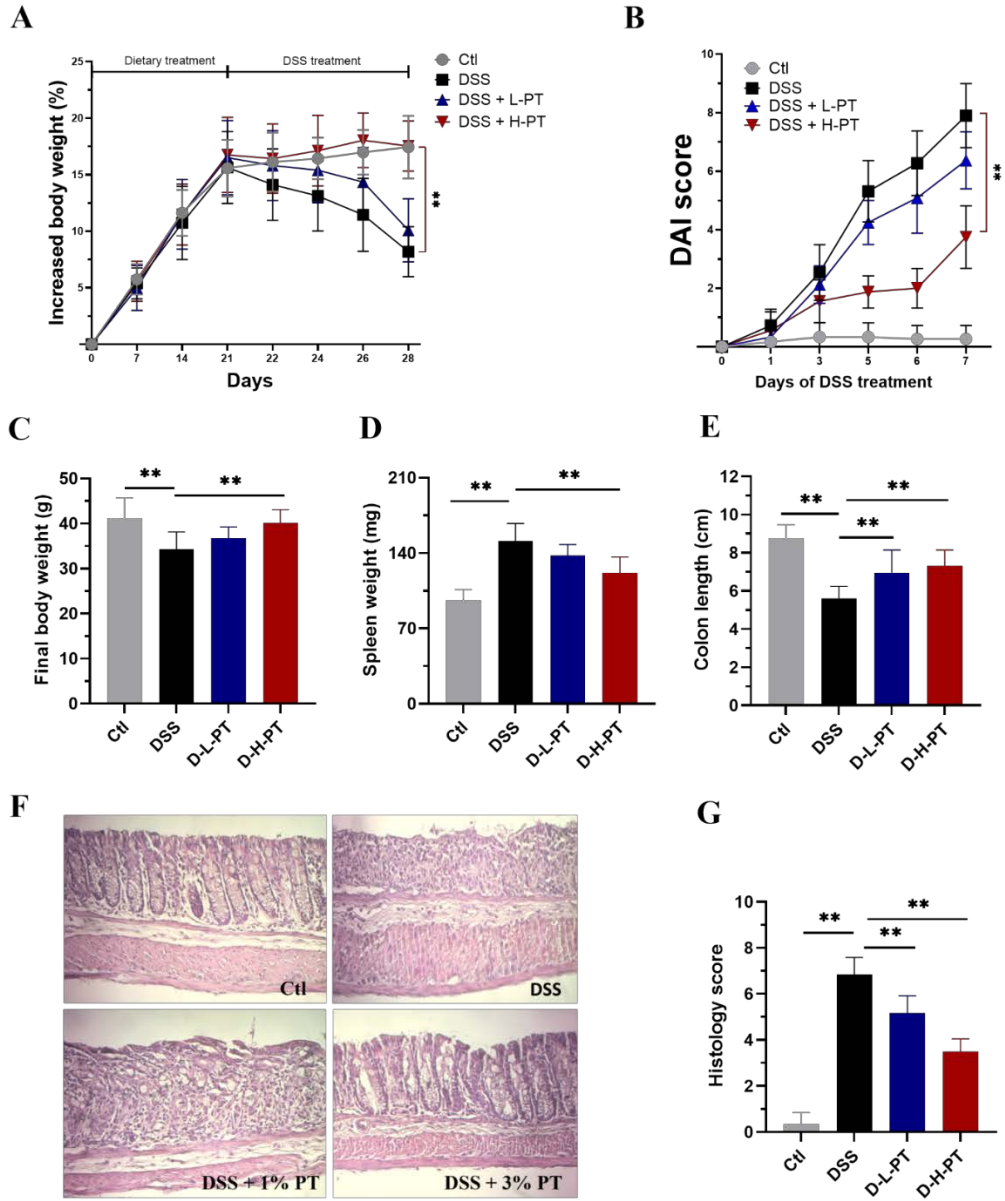


Figure.15. (A) The change of body weight during the entire experiments in different groups; (B) DAI scores in different groups; (C) Final body weight; (D) Spleen weight; (E) Colon length; (F) Representative image of H&E-stained colon (300 × magnification); (G) Histology scores of colonic damages. Data were presented as mean ± standard deviation (SD) (n = 8 - 10). ** indicate P < 0.01.

4.3.2 Intake of PT regulated the inflammatory-related protein and pro-inflammatory cytokines

The effects of PT on the productions of pro-inflammatory cytokines and proteins in colon mucosa were determined. The concentrations of TNF- α , IFN- γ , IL-1 β , IL-2 and IL-6 were all elevated in the DSS group. Oral administration of PT lowered these elevated cytokines in the colitis mice (Figure.16A). For instance, intake of 3% of PT slightly reduced the levels of TNF- α , IFN- γ , IL-1 β , IL-2 and IL-6 by 72.5, 87.9, 80.3, 66.1 and 70.4% in colitis mice, respectively, when compared to DSS group (Figure.16A). The protein levels of iNOS and COX-2 were elevated by 3.81- and 3.23-fold in the DSS group, in comparison to the control group. Intake of 3% PT lowered the iNOS and COX-2 protein levels by 67.4 and 56.6% in colitis mice, when compared to the DSS group (Figure.16B). Similar patterns were obtained in the protein levels of NF- κ B p50 and p65 (Figure.16B).

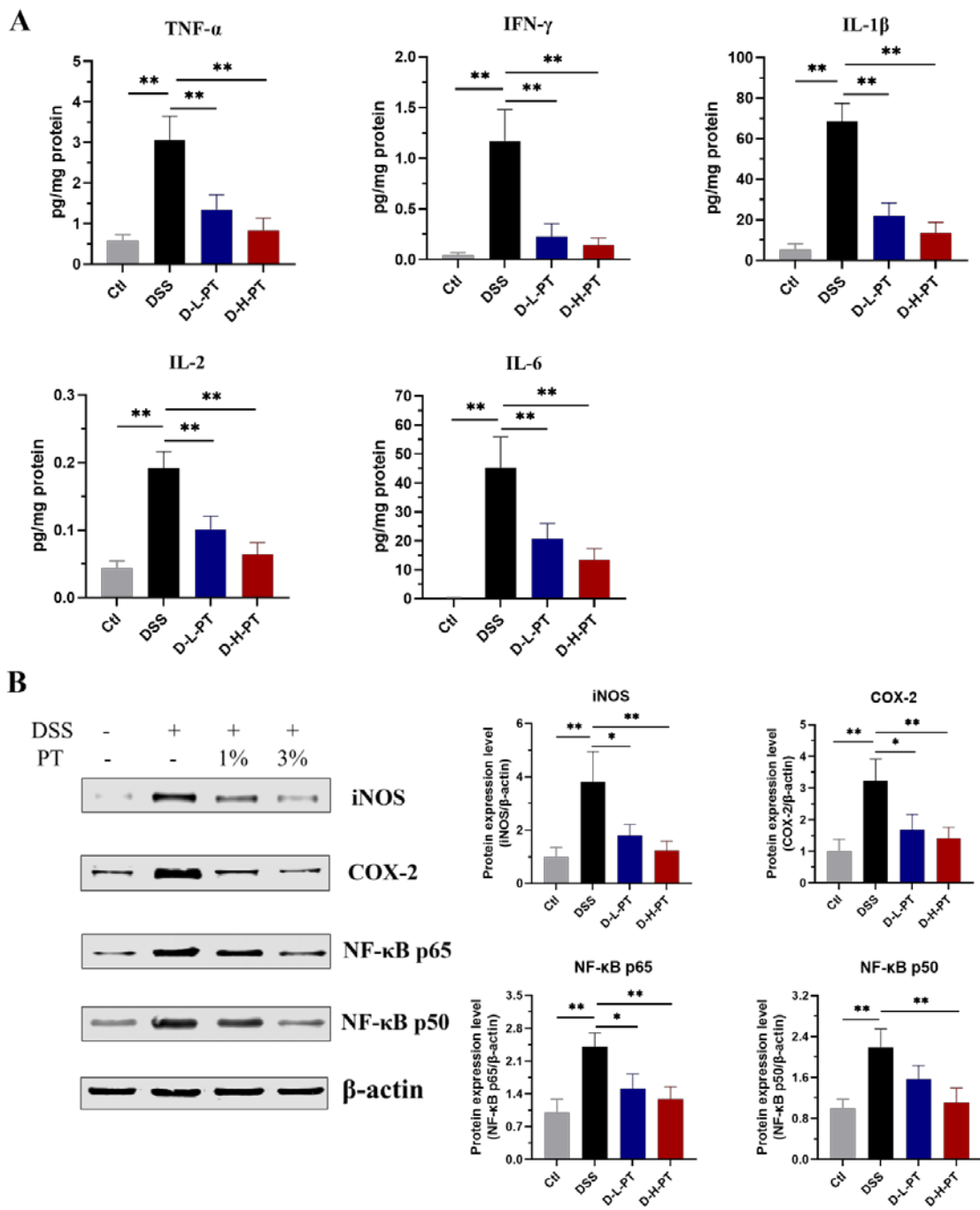


Figure.16. (A) Quantification of the pro-inflammatory cytokines in colonic mucosa (n = 8); (B) Western blotting analysis of inflammatory-related protein in colon mucosa (n = 8). Data were presented as mean \pm SD (n = 8 - 10). * indicate $P < 0.05$ and ** indicate $P < 0.01$.

4.3.3 Intake of PT shifted the gut microbiota diversity

The prebiotic effects of PT in gut microbiota were assessed by sequencing analysis. The α -diversity was evaluated by the operational taxonomic units (OTUs), Shannon and Chao 1 index. No apparent difference was found among the control, L-PT and H-PT groups (Figure.17A). Nevertheless, these indices were slightly decreased in the DSS group. Administration of PT shifted these indices forward to the control group in colitis mice (Figure.17A). The β -diversity, visualized by the principal coordinate analysis (PCoA), reflected the similarities of gut microbiota in different groups. Intake of PT did not cause apparent adverse effects among healthy group, while intake of PT slightly shifted the gut microbiota forward to the control group in colitis mice (Figure. 17B & 17C). Additionally, LEfSe analysis indicated that the proportion of *Enterococcaceae* and *Erysipelatoclostridium* were significantly elevated in the DSS group. In contrast, a higher percentage of *Lactobacillaceae*, *Lachnospiraceae*, *Bacilli* and *Robinsoniella* were identified in the control group. The D-H-PT group showed an elevated abundance of *Blautia*, *Turicibacter*, *Ruminococcaceae* and *Staphylococcus* (Figure.17D & 17E).

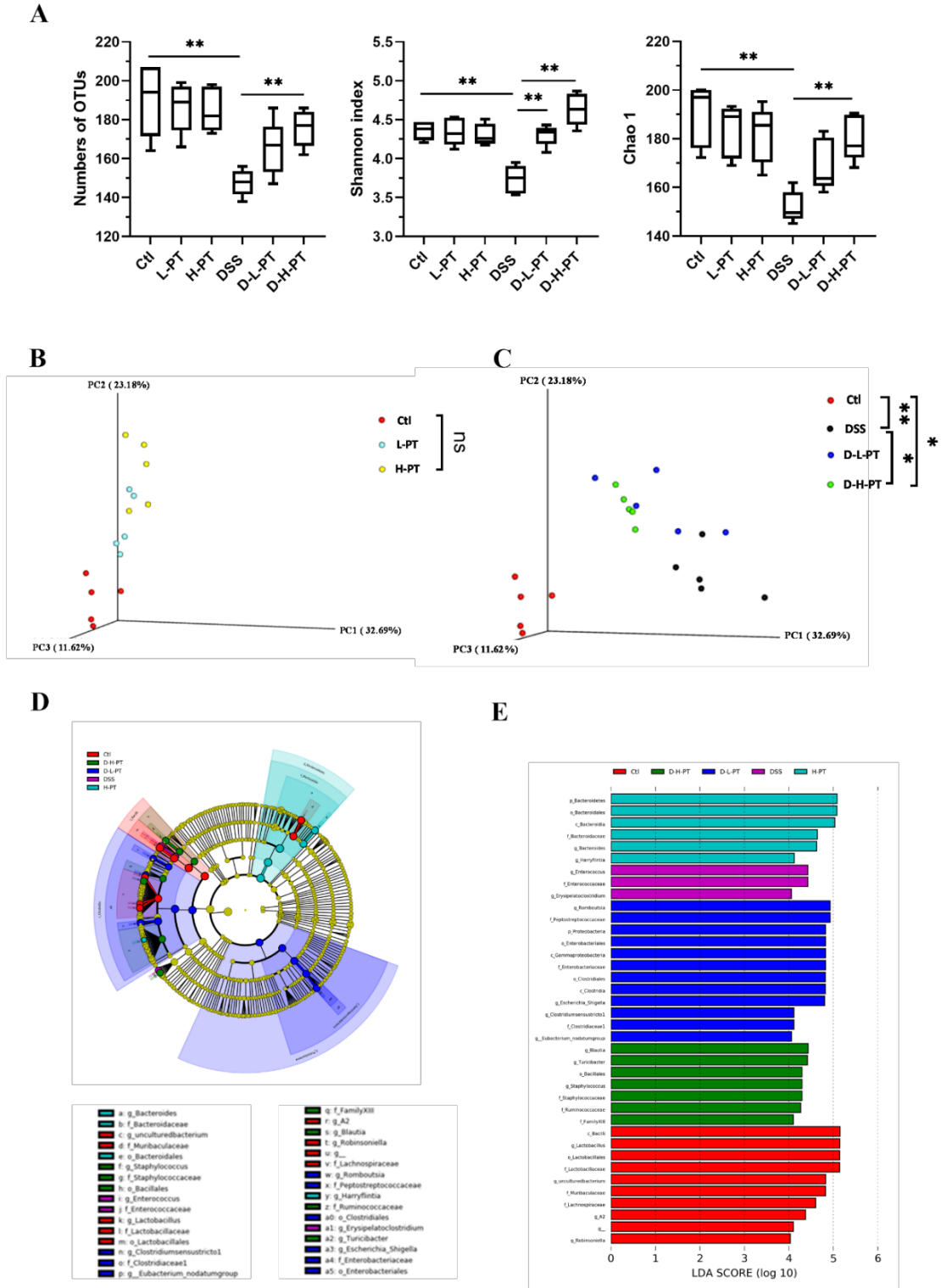


Figure.17. (A) PT alters the α -diversity of gut microbiota with various indices; (B) PT shifts the β -diversity of gut microbiota in healthy mice, visualized with PCoA plots; (C)

PT shifts the β -diversity of the gut microbiota in colitis mice, presented as PCoA plots; (D) Taxonomic cladogram obtained from LEfSe analysis ($\alpha < 0.1$); (E) Taxa with LDA score > 4 . Data were presented as mean \pm standard deviation (SD) (n =5). * indicate $P < 0.05$ and ** indicate $P < 0.01$.

4.3.4 Intake of PT reversed the gut microbiota structure

The alterations of bacteria at the phylum, family and genus levels from different groups were presented in Table.8. Overall, intake of PT did not cause adverse effects among healthy mice but reversed the relative abundance of bacteria forwarded to the control group in colitis mice (Table.8 & Figure.18). The proportion of Firmicutes was found to decrease in the colitis mice, and the abundance of Bacteroidetes was found to increase in the colitis mice, in comparison to the healthy mice (Figure.18A & 18B). It is noteworthy that administration of PT at 3% elevated the abundance of Firmicutes by 1.37-fold and suppressed the abundance of Bacteroidetes by 28.01% in colitis mice, when compared to the DSS group (Figure.18A & 18B). Concomitantly, the lowest ratio of Firmicutes to Bacteroidetes (F/B) was identified in the DSS group. Oral intake of 3% PT elevated the F/B by 1.91-fold in colitis mice, when compared to the DSS group.

At the genus levels, the abundance of *Enterococcus*, *Escherichia-shigella* and *Oscillibacter* were dramatically elevated by 9.48-, 10.57- and 45.7-fold in the DSS group, respectively, in comparison to the control group. Meanwhile, the proportion of *Blautia*, *Lactobacillus*, and *Roseburia* was sharply reduced by 50.21, 81.52 and 92.85% in the colitis mice, respectively, when compared to the healthy mice. Intake of 3% PT lowered the proportion of *Escherichia-shigella* and *Enterococcus* by 34.18 and 33.43% in colitis mice, respectively, in comparison to the DSS group (Figure.18C). Similar patterns were obtained in the *Oscillibacter* (Figure.18C). Additionally, administration of PT elevated the lowered

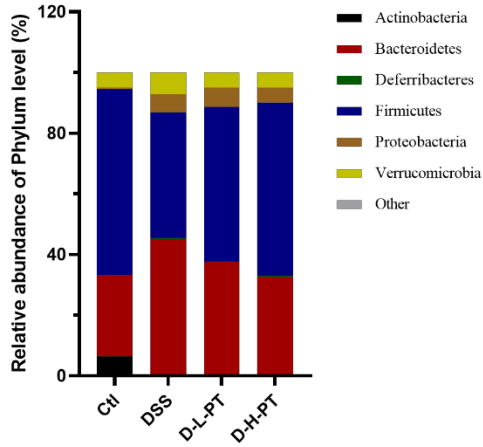
abundance of *Lactobacillus*, *Blautia* and *Roseburia* in colitis mice (Figure.18C). In line with these alterations, oral intake of the PT shifted the relative abundance of *Enterococaceae*, *Enterobacteriaceae*, *Lactobacillaceae* and *Lachnospiraceae* forward to the control group in colitis mice (Figure.18D).

Table.8. Relative abundance of gut microbiota at with significant differences in each group

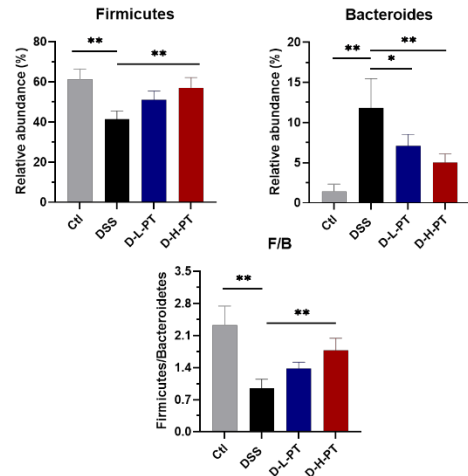
Relative abundance (%)	Ctl	L-PT	H-PT	DSS	D-L-PT	D-H-PT
Bacteroidetes	26.77 ± 3.49**	24.40 ± 4.29**	29.49 ± 7.20**	44.97 ± 4.82	37.23 ± 2.43	32.37 ± 2.51**
Firmicutes	61.27 ± 3.61**	64.32 ± 5.88**	58.80 ± 7.38**	41.49 ± 4.01	50.97 ± 2.03*	57.11 ± 4.06**
<i>Lactobacillaceae</i>	33.16 ± 5.95**	33.12 ± 6.45**	25.87 ± 2.05**	7.00 ± 2.61	11.67 ± 2.73	16.94 ± 4.36*
<i>Lachnospiraceae</i>	24.46 ± 2.39**	24.36 ± 5.18**	23.37 ± 4.98**	8.67 ± 1.17	11.68 ± 2.25	20.05 ± 3.38**
<i>Enterobacteriaceae</i>	0.88 ± 0.42**	0.61 ± 0.40**	0.08 ± 0.03**	8.97 ± 2.15	6.71 ± 2.29	5.75 ± 1.70*
<i>Ruminococcaceae</i>	2.57 ± 1.35**	3.84 ± 0.44**	3.41 ± 0.79**	7.91 ± 2.23	5.70 ± 1.71	2.89 ± 1.18**
<i>Enterococcaceae</i>	0.27 ± 0.13**	0.66 ± 0.38**	0.02 ± 0.01**	2.85 ± 0.97	1.58 ± 0.37*	1.71 ± 0.50*
<i>Staphylococcaceae</i>	0.05 ± 0.02**	0.17 ± 0.06**	0.13 ± 0.04**	1.24 ± 0.22	0.68 ± 0.35**	0.51 ± 0.25**
<i>Lactobacillus</i>	34.63 ± 8.45**	33.98 ± 5.17**	32.94 ± 4.05**	5.25 ± 1.39	13.39 ± 3.47	16.92 ± 4.05*
<i>Romboutsia</i>	0.69 ± 0.26**	0.55 ± 0.31**	0.36 ± 0.21**	4.13 ± 1.34	3.82 ± 0.76	1.79 ± 0.38*
<i>Escherichia-Shigella</i>	0.84 ± 0.62**	0.73 ± 0.36**	0.93 ± 0.22**	10.29 ± 4.11	6.98 ± 1.45	5.83 ± 1.19*
<i>Blautia</i>	6.83 ± 1.24**	6.34 ± 1.06**	5.97 ± 1.17**	2.45 ± 0.98	3.98 ± 1.19	5.22 ± 1.25*
<i>Enterococcus</i>	0.29 ± 0.15**	0.38 ± 0.14**	0.26 ± 0.07**	2.77 ± 1.03	2.09 ± 0.94	1.78 ± 0.31*
<i>Lachnospirillum</i>	2.43 ± 0.87**	2.46 ± 0.40**	2.31 ± 0.76**	1.10 ± 0.41	1.86 ± 0.29	1.77 ± 0.68*
<i>Staphylococcus</i>	0.62 ± 0.30**	0.68 ± 0.18**	0.70 ± 0.26**	3.07 ± 0.76	2.02 ± 0.96	1.39 ± 0.41**
<i>Ruminiclostridium</i>	0.19 ± 0.11**	0.32 ± 0.11**	0.31 ± 0.12**	1.40 ± 0.42	0.64 ± 0.13**	0.65 ± 0.15**
<i>Mucispirillum</i>	0.03 ± 0.02**	0.09 ± 0.02**	0.11 ± 0.08**	0.31 ± 0.09	0.18 ± 0.04*	0.19 ± 0.07*
<i>Oscillibacter</i>	0.03 ± 0.02**	0.07 ± 0.01**	0.04 ± 0.01**	1.37 ± 0.37	0.85 ± 0.17**	0.75 ± 0.23**
<i>Roseburia</i>	0.14 ± 0.06**	0.12 ± 0.06**	0.19 ± 0.03**	0.008 ± 0.004	0.02 ± 0.01	0.09 ± 0.02*

Results were presented as mean ± SD; * indicate P < 0.05, ** indicate P < 0.01, compared to the DSS group.

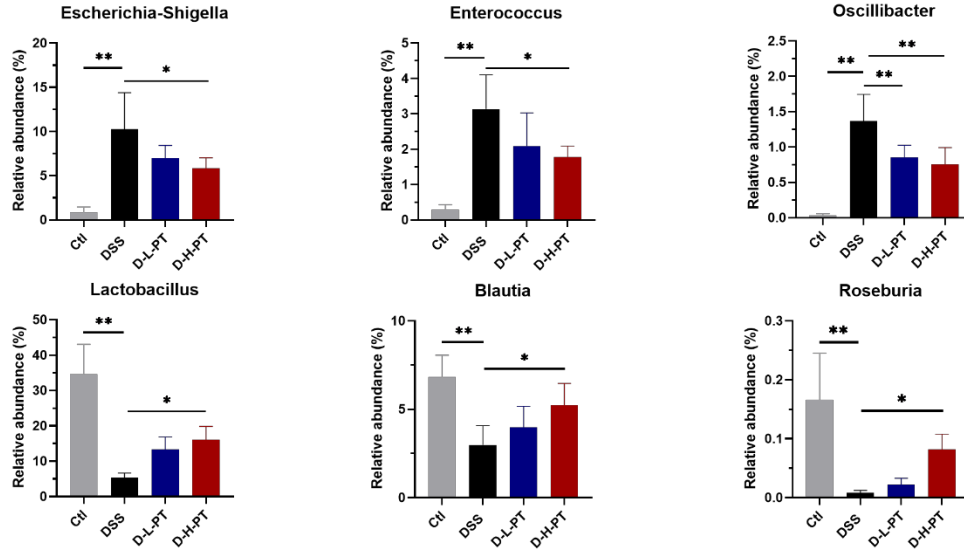
A



B



C



D

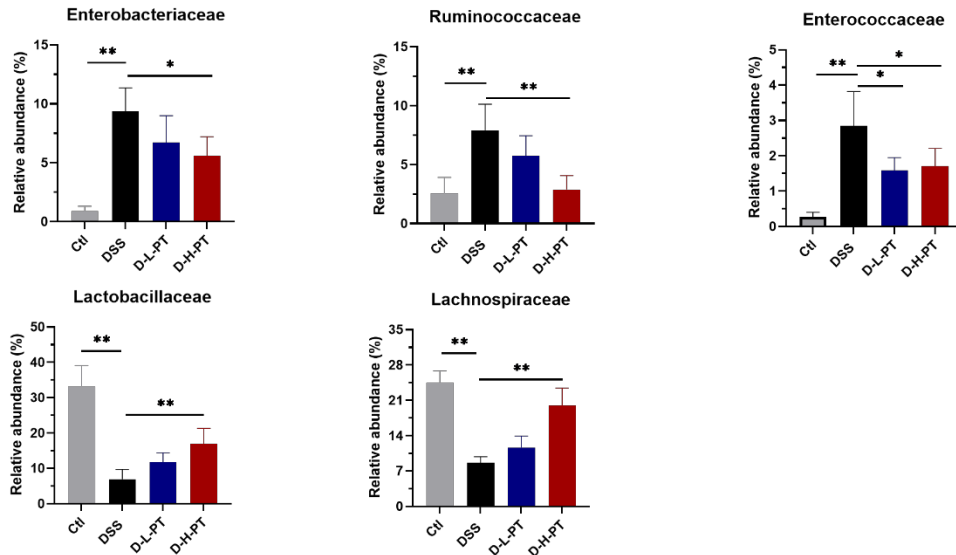


Figure.18. (A) The distribution of gut microbiota at the Phylum level in colitis mice (n = 5); (B) Relative abundance of Firmicutes and Bacteroidetes in colitis mice (n = 5); (C) Relative abundance of *Escherichia-Shigella*, *Enterococcus*, *Oscillibacter*, *Lactobacillus*, *Blautia* and *Roseburia* in colitis mice (n =5). (D) Relative abundance of *Enterobacteriaceae*, *Ruminococcaceae*, *Enterococcaceae*, *Lactobacillaceae* and *Lachnospiraceae* in colitis mice (n =5). Data were presented as mean \pm SD. * indicate $P < 0.05$ and ** indicate $P < 0.01$.

4.3.5 Intake of PT shifted the production of SCFAs and BAs

The concentrations of 6 SCFAs among all groups were quantified and listed in Table.1. The levels of total SCFAs were reduced by 66.88% in the DSS group, when compared to the control group. Administration of PT alleviated the dysregulated total SCFAs concentrations in colitis mice. For instance, intake of 3% PT enhanced the total SCFAs concentrations by 2.44-fold in colitis mice, in comparison to the DSS group (Table.9 & Figure.19A). Moreover, in line with the alteration of total SCFAs, intake of PT elevated the levels of propionic acids, butyric acids, acetic acid and valeric acids by 2.80-, 2.68-, 2.50-, and 2.34-fold in colitis mice, in comparison to the DSS group (Figure.19A).

The variation of the 17 identified BAs is listed in Table.9. The total primary BAs were significantly decreased, and the total secondary BAs were slightly increased in the DSS group. Nevertheless, Intake of PT reversed the total primary and secondary BAs forward to the control group in colitis mice (Table.9). The concentrations of T- α -MCA and T- β -MCA, two of the conjugated primary BAs, were significantly elevated by 70.65- and 5.45-fold in the DSS group, respectively, when compared to the control group. Moreover, the raised T- α -MCA and T- β -MCA were reduced by the administration of PT in colitis mice, when compared to the DSS group (Figure.19B). Moreover, the concentrations of unconjugated primary BAs (α -MCA and β -MCA) and secondary BAs (MDCA, DCA, LCA,

T-DCA and T-LCA) were found to decrease in the DSS group. The concentrations of these BAs were enhanced by the administration of PT in colitis mice, in comparison to the DSS group (Figure.19B & 19C). Notably, intake of 3% PT enhanced the levels of LCA and DCA in colitis mice by 1.96- and 4.10- fold, respectively, when compared to the DSS group (Figure.19C).

Table.9. SCFAs and BAs content in different groups treated by PT.

SCFAs or BAs ($\mu\text{g/g}$)	Ctl	L-PT	H-PT	DSS	D-L-PT	D-H-PT
Acetic acid	1745.71 \pm 313.63**	1881.39 \pm 439.95**	1790.96 \pm 204.18**	533.59 \pm 174.55	1012.74 \pm 268.57	1334.48 \pm 300.87**
Propionic acid	822.37 \pm 298.82**	719.43 \pm 173.67**	722.34 \pm 117.36**	250.61 \pm 87.47	513.98 \pm 183.38	702.56 \pm 185.70*
Butyric acid	819.29 \pm 280.50**	626.54 \pm 146.23**	679.87 \pm 228.24**	259.05 \pm 98.35	495.02 \pm 115.47	699.19 \pm 175.51**
Iso-butyric acid	31.47 \pm 12.50	34.26 \pm 8.86	32.28 \pm 13.27	23.18 \pm 7.24	29.27 \pm 3.39	36.19 \pm 8.38
Valeric acid	212.04 \pm 39.08**	189.60 \pm 26.80**	191.67 \pm 44.35**	71.70 \pm 17.97	120.89 \pm 32.42	167.82 \pm 37.20**
Iso-valeric acid	105.85 \pm 15.59	101.10 \pm 23.36	100.28 \pm 18.36	99.33 \pm 15.96	98.53 \pm 22.30	97.67 \pm 16.03
Total SCFAs	3736.75 \pm 691.67**	3552.36 \pm 636.48**	3517.43 \pm 330.01**	1237.49 \pm 377.91	2270.93 \pm 504.01**	3031.38 \pm 556.28**
α -MCA	117.82 \pm 47.75**	115.14 \pm 48.52**	111.27 \pm 47.07**	22.49 \pm 8.90	63.36 \pm 20.60	106.17 \pm 40.18**
β -MCA	457.32 \pm 115.66**	452.40 \pm 111.92**	450.44 \pm 109.49**	204.02 \pm 54.33	254.86 \pm 35.17	340.87 \pm 46.54**
CA	222.47 \pm 53.08**	218.65 \pm 55.21**	216.63 \pm 48.53**	687.62 \pm 95.25	511.45 \pm 97.76*	360.57 \pm 65.44**
T- α -MCA	6.93 \pm 1.64**	6.68 \pm 1.42**	6.59 \pm 1.47**	82.57 \pm 34.78	17.14 \pm 6.79**	15.32 \pm 6.76**
T- β -MCA	136.62 \pm 45.05**	134.15 \pm 43.99**	128.56 \pm 44.17**	745.93 \pm 114.19	435.05 \pm 68.21**	329.82 \pm 46.54**
T-CA	109.82 \pm 34.63	108.37 \pm 33.90	104.40 \pm 32.72	170.51 \pm 43.43	157.21 \pm 41.50	133.02 \pm 33.75
CDCA	6.89 \pm 2.55*	6.70 \pm 2.65	6.67 \pm 2.62	2.98 \pm 1.72	4.17 \pm 1.32	4.60 \pm 1.39
T-CDCA	5.05 \pm 1.24	4.93 \pm 1.16	4.79 \pm 1.21	2.66 \pm 0.92	4.14 \pm 1.43	4.87 \pm 1.30
Total primary BAs	1062.96 \pm 161.39**	1047.06 \pm 158.62**	1029.37 \pm 155.99**	1918.85 \pm 144.13	1447.43 \pm 129.05**	1295.28 \pm 62.89**
T- ω -MCA	38.26 \pm 8.25*	38.74 \pm 6.78*	35.63 \pm 7.51*	73.20 \pm 17.57	101.73 \pm 19.33*	80.93 \pm 11.26
ω -MCA	411.23 \pm 23.65	408.19 \pm 23.72	419.93 \pm 24.89	491.51 \pm 67.52	561.15 \pm 53.55	521.87 \pm 46.52
MDCA	42.23 \pm 13.23**	40.93 \pm 12.85**	38.63 \pm 10.90**	13.55 \pm 5.36	21.83 \pm 9.27	34.33 \pm 11.95**
UDCA	12.18 \pm 5.26	11.04 \pm 4.23	10.61 \pm 3.32	6.29 \pm 1.83	7.70 \pm 2.41	10.23 \pm 4.21
DCA	274.75 \pm 57.15**	273.02 \pm 56.88**	260.66 \pm 56.40**	61.62 \pm 22.27	82.81 \pm 30.66	252.80 \pm 35.74**
LCA	92.05 \pm 20.52**	90.44 \pm 20.82**	87.15 \pm 24.41**	43.81 \pm 8.64	67.75 \pm 11.45	86.18 \pm 23.71**
T-UDCA	31.94 \pm 14.08	31.21 \pm 13.27	29.65 \pm 13.71	14.97 \pm 7.14	20.42 \pm 7.91	23.70 \pm 7.01
T-DCA	53.69 \pm 23.22**	51.85 \pm 22.95**	49.07 \pm 23.14**	9.22 \pm 3.33	13.96 \pm 7.11	25.40 \pm 7.30*
T-LCA	15.48 \pm 3.87**	14.87 \pm 3.95*	14.52 \pm 3.44*	6.37 \pm 1.91	13.56 \pm 3.89	14.31 \pm 3.88*
Total secondary BAs	972.25 \pm 70.76**	960.32 \pm 69.34**	945.85 \pm 77.81**	720.58 \pm 80.48	890.96 \pm 51.55	1049.77 \pm 46.83**

Results were presented as mean \pm SD; * indicate P < 0.05, ** indicate P < 0.01, compared to the DSS group.

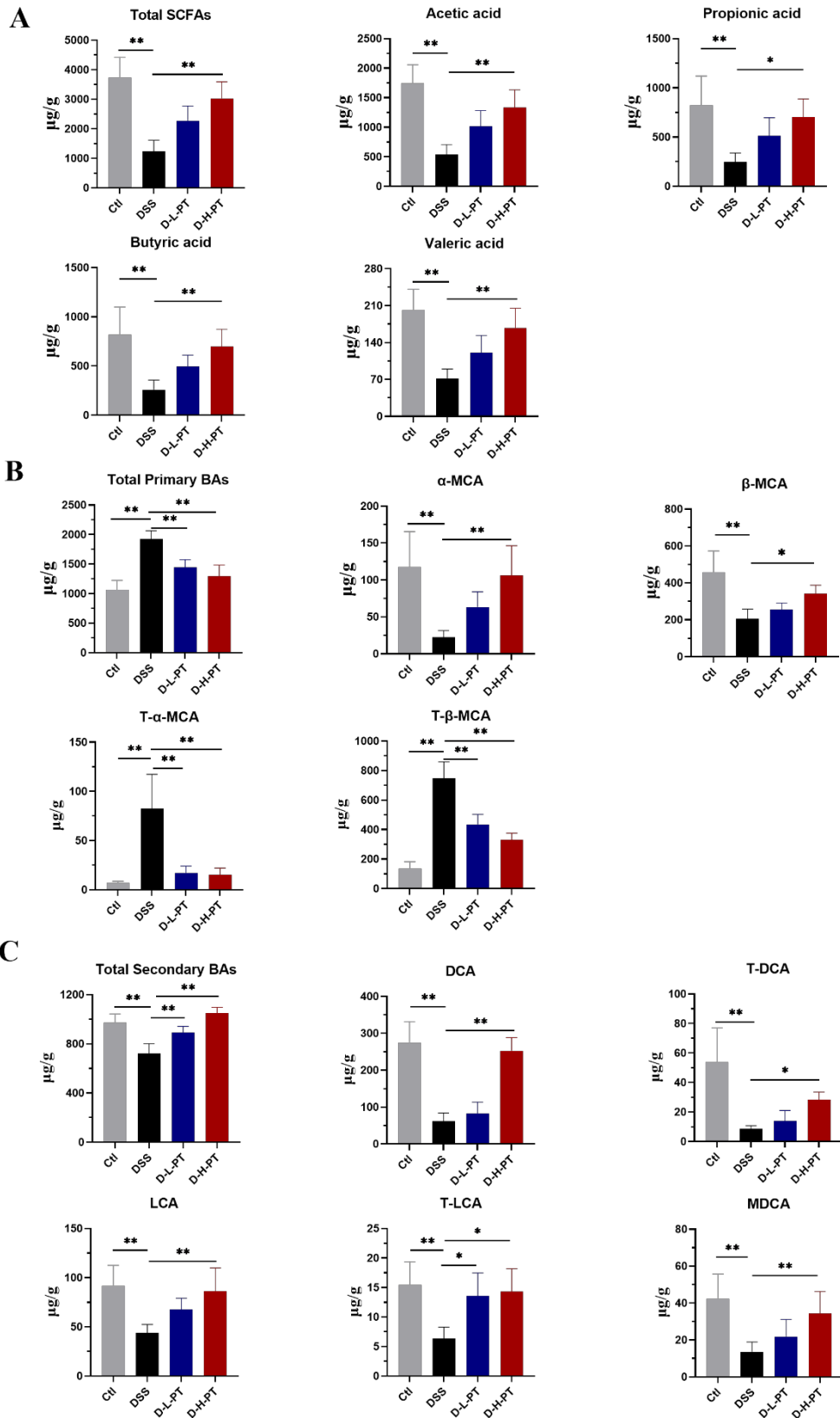


Figure.19. Concentrations of SCFAs (A), primary BAs (B) and secondary BAs (C) in different groups of mice. Results were presented as mean \pm SD. * indicate $p < 0.05$, ** indicate $P < 0.01$.

4.3.6 Correlation analysis between the gut microbiota and colitis indices

According to the abovementioned findings, colitis mice were associated with the improper dysregulation of pro-inflammatory cytokines, SCFAs and BAs, as well as improper alteration of the relative abundance of identified gut microbiota. Pearson correlation was conducted to evaluate the relationship between the gut microbiota and colitis parameters (Figure.20). The alteration of SCFAs and secondary BAs were positively with bacteria from the family of *Lactobacillaceae* and *Lachnospiraceae*, especially for the *Lactobacillus*, *Blautia* and *Roseburia*, and inversely with the potential harmful bacteria, containing *Staphylococcus*, *Oscillibacter* and *Escherichia-Shigella*. In contrast, the alteration of pro-inflammatory cytokines was negatively associated with the potential beneficial bacteria and positively correlated with the potential harmful bacteria.

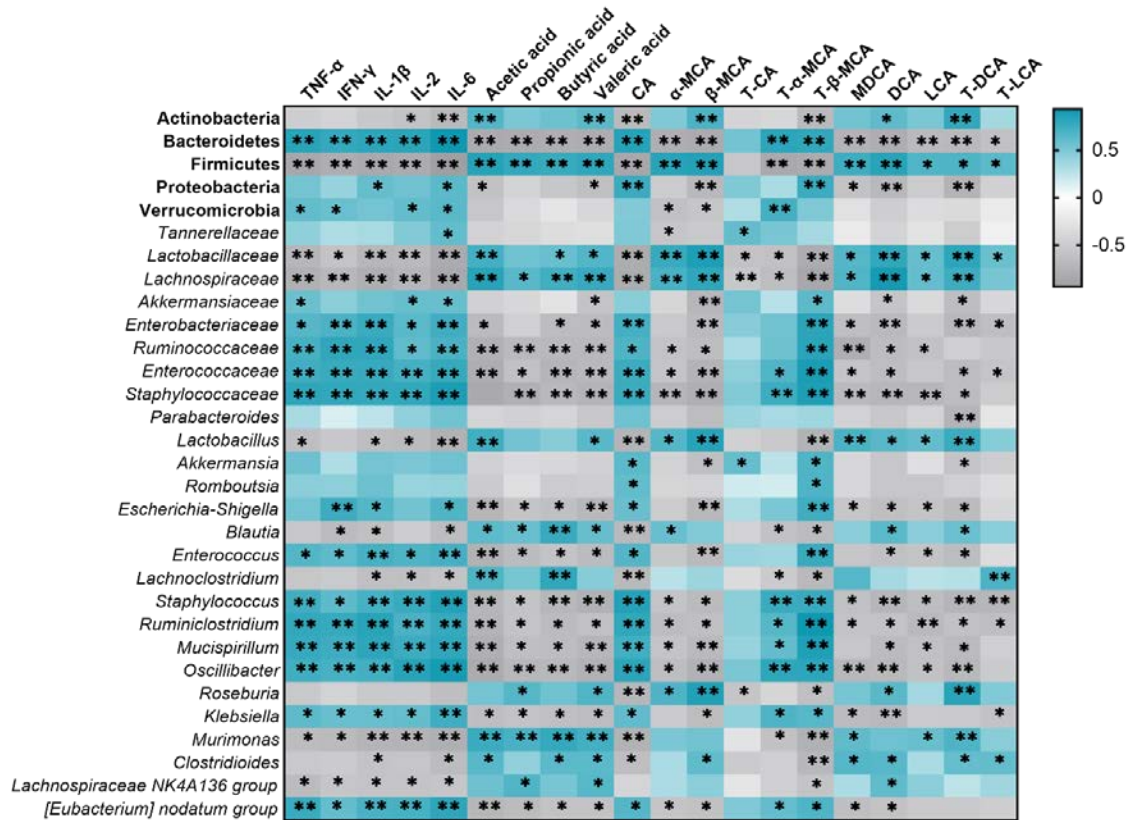


Figure.20. Heatmap of the correlation between the bacterial and cytokines or SCFAs or BAs. Results were presented as mean \pm SD. * indicate $p < 0.01$, ** indicate $P < 0.001$.

4.4 Discussion

The pathogenesis of intestinal inflammation is unclear, which was found to increase the risk of colon cancer [247]. Conventional drug therapy may lead to various distinct side effects that yield a higher recurrence rate [217]. Recently, the dietary intervention for UC has received more attention [248]. Bioactive components isolated from red seaweed are known for their biological properties against various chronic diseases [80, 96, 249]. However, the knowledge about the impacts of whole PT on intestinal health is still scattered. The dose in the current study were 1% and 3% in the mouse diet, equivalent to around 3.6 g and 10.8 g of dried powder per person per day [218]. In the current study, intake of PT

significantly ameliorated the physical signs of colitis, including bodyweight, diarrhea, rectal bleeding, colon length and spleen weight, and repairing the colonic histology damage. Additionally, the imbalance of cytokines is vital in inflammatory responses and can partially affect the severity of intestinal inflammation [250]. The genes of these cytokines have binding sites for the nuclear factor- κ B (NF- κ B), and the activation NF- κ B pathway greatly influences colitis's pathogenesis [220]. In the current study, intake of PT was also reported to down-regulation of proinflammatory-cytokines and proteins in colon mucosa. These findings indicated that oral administration of PT alleviated the severity of colitis via restoration of immune homeostasis.

Gut microbiota is vital in the pathogenesis of IBD and the correlation between the gut microbiota and IBD was explained in a study in germ-free mice [251]. In the current study, intake of PT did not alter the gut microbiota diversity among healthy mice. Meanwhile, the gut microbiota diversity was declined in the DSS group. Intake of PT was found to enhance the declined gut microbiota diversity in colitis mice. Additionally, An elevation of potential harmful bacteria and a reduction of potential beneficial bacteria were associated with colonic inflammation [252, 253]. Bacteroidetes and Firmicutes are two major phyla and the ratio of F/B is vital in the maintaining homeostasis [230]. In the present study, the ratio of F/B was declined in the DSS group, and intake of PT alters the ratio of F/B in colitis mice forwarded to the control group. While, at the genus levels, *Lactobacillus*, a beneficial bacteria belonging to the family of *Lactobacillaceae*, was found to have stronger anti-inflammatory properties in various studies [254]. Oral administration of *Lactobacillus* was found to against IBD in various murine models [255, 256]. *Blautia* and *Roseburia*, from the family of *Lachnospiraceae*, were found to against inflammation and regulate gut

microbial ecology [257, 258]. In contrast, *Escherichia-shigella* and *Enterococcus*, two potentially harmful bacteria belonging to the *Enterobacteriaceae* and *Enterococcaceae*, respectively, were found to induce IBD in a murine model [235, 236]. *Oscillibacter* were also found have a positive correlation with the severity of IBD [259]. In the current study, intake of PT was found to upregulation of *Lactobacillus*, *Blautia* and *Roseburia*, as well as downregulation of *Enterococcus*, *Escherichia-shigella* and *Oscillibacter* in colitis mice forwarded to the control group. Additionally, these alterations were also validated by the LEfSe analysis at OTU level, where the dominant bacteria were different among all groups.

The alteration of gut microbiota-derived metabolites, especially for the SCFAs and BAs, are disturbed in IBD patients and experimental colitis [204, 260, 261]. SCFAs are generated from the fermentation of the dietary fiber and proteins, which are metabolized by the intestinal microbiota [262]. Meanwhile, the anti-colitis properties of SCFAs have also been reported in various animal studies [263, 264]. In addition, gut microbiota dysbiosis significantly impacts the biotransformation of BAs in the large intestine, which is accompanied by the reduction of secondary BAs, and accumulation of the conjugated primary BAs. The bacteria, including *Bacteroides*, *Clostridium*, *Ruminococcus*, and *Lactobacillus* is vital in the BAs transformation [222, 223]. Moreover, a lower concentration of secondary BAs, especially for the LCA and DCA, were found in the IBD patients and colitis mice [223]. In the current study, the dysregulated SCFAs and BAs in colitis mice were partially reversed by the administration of PT. Additionally, further correlation analysis suggested that the regulation of SCFAs and BAs were strongly associated with the alteration of gut microbiota, particularly for the bacteria from the family of *Lactobacillaceae* and

Lachnospiraceae. These findings suggested that oral administration of PT altered the gut microbiota dysbiosis in colitis mice.

CHAPTER 5

CONCLUSION AND FUTURE PERSPECTIVES

Seaweeds may be used as an alternative medicine for chronic diseases. Seaweed and their isolated bioactive compounds profoundly modulate the gut microbiota composition and structure. These alterations not only promote colon health in disease-free populations but may also alleviate the severity of gut microbiota-associated chronic diseases. Bioactive compounds from seaweeds have been reported to exert protective effects on IBD and alter IBD-related gut microbiota in *in vitro* and *in vivo* studies. These findings provided a fundamental understanding of the potential benefits of seaweeds against IBD in humans. Nevertheless, a deeper investigation focusing on the role of seaweeds against IBD is necessary. Firstly, large amounts of bioactive compounds from seaweeds showed protective effects against colitis via restoring immune homeostasis. In contrast, their protective effects against IBD via alleviating the dysbiosis of gut microbiota and enhancing their metabolite profiles have been largely neglected in these studies. Secondly, isolated bioactive compounds are just parts of seaweed. Few reports have targeted the effects of the administration of whole seaweed and their effects on gut microbiota. The relationship among the whole seaweed, IBD and gut microbiota is unclear. Potential antagonistic and/or synergistic effects may be produced when different compounds interact in the whole seaweed, which warrants further investigation. A more incisive understanding of the mechanisms of action behind whole seaweed and IBD will provide a scientific basis for developing effective and safe diet-based strategies to prevent IBD using whole seaweed. Thirdly, controlled human intervention trials are necessary to elucidate how seaweed protects IBD, which may be the endpoint step to illustrate the health benefits of seaweed.

This cell study, for the first time, elucidated the composition of polyphenols-rich components from three edible seaweeds, *L. japonica*, *P. tenera* and *U. lactuca*, and we further investigated their efficacy and mechanisms against inflammation and colon cancer in cell studies. We found that EPCs and NEPCs exerted potent inhibitory effects in activated macrophages via suppressing pro-inflammatory cytokines and enzymes and activating antioxidant enzymes. At the same time, they lowered the proliferation of HCT116 cells by inducing cell cycle arrest and cell apoptosis. The novel extracts of edible seaweed may offer a safe, inexpensive and efficacious dietary strategy to prevent colon cancer in humans, especially in individuals with chronic inflammation. Further work will comprehensively evaluate the anti-inflammatory capacities and anticancer properties of polyphenols-rich components from edible seaweeds in animal and human studies.

The animal study indicated that supplementation of LJ and PT were found to attenuate colonic inflammation in the DSS-induced murine model, evidenced by repairing colonic injuries, inhibiting the pro-inflammatory cytokines and inflammatory-related proteins. Moreover, the anti-colitis effects of LJ and PT were associated with their properties to alleviate the gut microbiota dysbiosis, which was suggested by the alteration of certain identified microbiota, as well as the regulation of SCFAs and BAs. Collectively, our results provide a rationale for the utilization of edible seaweeds as a potential prebiotic for the prevention of colonic inflammation.

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