



Research Article

The *in vitro* and *in vivo* antioxidant activities of *Sargassum pallidum* ethanol extract and its effect on gut microbiota modulation

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Abstract: *Sargassum pallidum* (*S. pallidum*), a traditional Chinese herb, is a homology of medicine and food that is widely used daily. This study focused on the antioxidant activity and modulation of the gut microbiota by *S. pallidum* ethanol extract (SPEE). The results of component determination showed that SPEE contained flavonoids (0.398 g/100 g) and polyphenols (0.193 g/100 g). SPEE effectively eliminated 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonate) (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and hydroxyl and superoxide radicals *in vitro*; reduced the reactive oxygen species (ROS) and malondialdehyde (MDA) contents; and increased the glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), and catalase (CAT) enzymatic activities in UVB-irradiated HaCaT cells, which indicated its *in vitro* antioxidant activity. In addition, the healthy mice that received SPEE treatment presented a reduced spleen index and MDA content and increased GSH-Px enzymatic activity, which indicated its *in vivo* antioxidant activity. Furthermore, 16S rRNA gene sequencing of fecal samples revealed that SPEE modulated the gut microbiota in mice, decreasing the abundance of harmful bacteria such as *Helicobacter* while increasing the abundance of beneficial bacteria such as *Bifidobacterium* and *Akkermansia*. This study systematically confirmed the antioxidant activity of SPEE, clarified its ability to modulate the gut microbiota, and elucidated the relationship between its antioxidant activity and the gut microbiota, laying a foundation for in-depth research and wider application of *S. pallidum* in antioxidants.

Keywords: *Sargassum pallidum*; ethanol extract; antioxidant; gut microbiota

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1 Introduction

Numerous studies have demonstrated the link between the overproduction of free radicals and various health issues, such as obesity, cardiovascular disease, and cancer^[1]. Typically, cellular processes produce reactive oxygen species (ROS), which are beneficial within certain limits but may become harmful when this balance is disrupted by external stressors such as high temperatures or radiation^[2]. In response, exogenous antioxidants mitigate these effects by neutralizing free radicals, chelating metal ions, and enhancing endogenous antioxidant enzymes^[3]. While traditional antioxidants, often derived from chemical synthesis, can pose stability and toxicity issues, natural plant antioxidants provide safer and more stable options.

Sargassum pallidum (Turn.) C. Agardh, a significant and typical edible and medicinal brown alga from the *Sargassum* family, is

found primarily across East Asia, including China and South Korea. Numerous studies have indicated that *S. pallidum* is rich in various bioactive substances, including polysaccharides, polyphenols, terpenoids, essential fatty acids, and minerals, which contribute to its antioxidant, antibacterial, antitumor, and hypoglycemic activities^[4]. Previous studies reported that polysaccharide nanoparticles from *S. pallidum* enhanced antioxidant activity^[5], whereas another study prepared four polysaccharide fractions from *S. pallidum* and reported that different fractions presented different antioxidative activities and bioactivities^[6]. However, these studies focused mainly on the biological activities of polysaccharides derived from *S. pallidum*, while the specific antioxidant capabilities of other components remain to be investigated.

The gut microbiota is closely related to antioxidation. On the one hand, many diseases associated with oxidative stress, such as

diabetes^[7], cardiovascular disease^[8], and Alzheimer's disease^[9], have been shown to correlate with changes in the composition and metabolism of the gut microbiota. On the other hand, the mechanisms by which the gut microbiota, such as *Clostridium* and *Lactobacillus*, enhances antioxidant capabilities have been partially elucidated^[10]. Therefore, exploring the antioxidative mechanisms of *S. pallidum* through the gut microbiota and identifying key antioxidative bacteria are both necessary and feasible.

This study aims to systematically evaluate the *in vitro* and *in vivo* antioxidant activities of SPEE and explore its modulatory effects on the gut microbiota. Furthermore, it seeks to identify specific bacteria related to antioxidation and to preliminarily elucidate the mechanisms linking its antioxidant activity with the gut microbiota.

2 Materials and methods

2.1 Materials and chemicals

S. pallidum ethanol extract (SPEE) was purchased from Ningbo Lihua Pharmaceutical Co. Ltd. (Ningbo, Zhejiang, China). Dimethyl sulfoxide (DMSO), Dulbecco's Modified Eagle's Medium (DMEM), and carboxymethyl cellulose sodium (CMC-Na) were purchased from Sigma-Aldrich, Inc. (St. Louis, MO, USA). Fetal bovine serum was purchased from HyClone Co., Ltd. (Logan, UT, USA), and antibiotics (100 U/mL penicillin and 0.25 µg/mL streptomycin) were obtained from Thermo Fisher Scientific, Inc. (Waltham, MA, USA). Trypsin-EDTA solution was purchased from Shanghai Yuanye Biotechnology Co., Ltd. (Shanghai, China). Cell Counting Kit-8 (CCK-8), total antioxidant capacity (T-AOC), DPPH free radical scavenging capacity, hydroxyl free radical inhibition, superoxide anion, ROS, malondialdehyde (MDA), glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), and catalase (CAT) assay kits were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, Jiangsu, China). The BCA protein assay kit was obtained from Beyotime Biotechnology Co. Ltd. (Shanghai, China). All other reagents were of analytical grade and were commercially available.

2.2 Preparation of SPEE

The extract from *S. pallidum* was prepared according to the methods of Xie *et al.* with some modifications^[11]. The dried powder of *S. pallidum* was soaked with 95% aqueous ethanol at a ratio of 1:40 (*m/v*) for 12 h and extracted ultrasonically at 50 °C for 30 min. The mixtures were subsequently centrifuged, and the residues were re-extracted twice via the same process. After filtration and residue removal, the extract was concentrated and freeze-dried to obtain SPEE. It was stored at -80 °C until further use.

2.3 SPEE content determination

In agreement with previous work by Chen *et al.*^[12], the flavonoid content in SPEE was measured via the chemical coloration method. Following the methods of Skerget *et al.*^[13], the total polyphenol content in SPEE was quantified via the Folin-Ciocalteu assay.

2.4 In vitro antioxidant activity analysis

In the experimental design of Wang *et al.*^[14], SPEE was dissolved in anhydrous ethanol at a concentration of 100 mg/mL to create a stock solution. Using anhydrous ethanol, this stock was

then diluted to 1.00, 0.75, 0.50, 0.25, and 0.10 mg/mL. The *in vitro* scavenging activities of SPEE on 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonate) (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), hydroxyl, and superoxide radicals were evaluated by corresponding assay kits. As a positive control, ascorbic acid (Vc) was dissolved in sterilized water at the same concentration gradient.

2.5 Cell experiment

2.5.1 Cell culture

HaCaT cells were purchased from Beijing Beina Chuanglian Biotechnology Co., Ltd. (Beijing, China). They were grown in DMEM containing 10% fetal bovine serum and 1% antibiotics (100 U/mL penicillin and 0.25 µg/mL streptomycin). The cells were maintained at 37 °C in a humidified environment with 5% CO₂. Once the cell cultures exceeded 90% confluence, they were passaged, digested with a 0.05% trypsin-EDTA solution, and then counted for further analysis.

2.5.2 Cytotoxicity assays

HaCaT cells were plated into 96-well microplates at a density of 10,000 cells per well and incubated at 37 °C for 24 h. First, the toxicity of SPEE on HaCaT cells was evaluated. SPEE was dissolved in DMSO and then diluted with DMEM to different concentrations (25, 50, 100, 200, 500, and 1000 µg/mL), ensuring that the final DMSO concentration was 0.5%. The culture medium was removed, and the cells were incubated with each SPEE concentration for 24 h. Afterward, the medium was discarded, and cell viability was measured via CCK-8 assays, according to the manufacturer's protocol. The absorbance was measured at 450 nm with a microplate reader (iMark, Bio-Rad, USA). Results were expressed as a percentage of the control group (untreated) and averaged across six experiments. Second, the toxicity of UVB irradiation on HaCaT cells was assessed. In accordance with the methods of Han *et al.*^[15], the culture medium was removed, PBS was added to cover the cells, and the cells were exposed to UVB irradiation at doses of 20 or 40 mJ/cm², while the control wells were shielded with aluminum foil. After UVB irradiation, the original culture medium was returned to the cells, and the cells were incubated for various periods (2, 8, 12, 16, and 20 h). Cell viability was then evaluated via CCK-8 assays. The results were expressed as a percentage of the control group (without UVB irradiation) and averaged across six experiments.

2.5.3 SPEE treatment of UVB-irradiated HaCaT cells

In section 2.5.2, HaCaT cells were divided into control, model, and SPEE groups (25, 50, and 100 µg/mL), with six replicates each. The control and model groups were cultured in DMEM for 16 h, while the SPEE groups were treated with varying concentrations of SPEE for the same duration. Post-treatment, the medium was replaced with PBS before the cells were exposed to UVB irradiation at 20 or 40 mJ/cm², with the control group shielded with aluminum foil. After irradiation, the original medium was reinstated, and the cells were incubated for 8 h. The impact of SPEE on the viability of UVB-irradiated HaCaT cells was assessed via CCK-8 assays, with the results expressed as a percentage of those of the control group.

2.5.4 Determination of intracellular oxidation indicators

According to 2.5.3, HaCaT cells were divided into control, model, and SPEE groups (25 and 50 µg/mL), with six replicates

each. Except for the UVB irradiation dose of only 20 mJ/cm², the operation was the same as that described in section 2.5.3. The medium was subsequently removed, and the ROS level was determined via an ROS assay kit according to the manufacturer's protocol. The results were observed under a fluorescence microscope (Nikon, Tokyo, Japan).

The processing steps for the cell experiment conditions were the same as those described above. The medium was subsequently removed, and the cells from each group were collected and washed twice with PBS. After centrifugation, the supernatants were discarded, and the cell pellets were homogenized in physiological saline at a 1:9 ratio (w/v) to create whole homogenates. These homogenates were then centrifuged at 12,000×g for 20 min at 4 °C, after which the supernatants were collected. A portion of the supernatant was used for protein quantification via a BCA protein assay kit. The levels of MDA, GSH-Px, SOD, and CAT in the supernatants were determined with commercial kits according to the manufacturer's instructions. The MDA levels were expressed in nmol/mg prot, whereas the other values were reported in U/mg prot.

2.6 Animal trial

2.6.1 Animal experiment design

Based on previous studies^[16-19], thirty-six male ICR mice (weighing 25 ± 2 g) were obtained from the Laboratory Animal Center of Zhejiang Province (Hangzhou, Zhejiang, China). The protocol was approved by the Ethical Committee of Experimental Animal Care at Ningbo University (Ningbo, Zhejiang, China) based on the standard guidelines and rules of this institution (NBU12157). After one week of acclimatization, the mice were randomly divided into control, low-dose (LD), and high-dose (HD) groups. Their baseline body weights (0 weeks) were recorded. SPEE was dissolved in a 0.5% CMC-Na solution (with ultrapure water as the solvent). The LD group received 100 mg/(kg·day) SPEE via gavage, the HD group received 200 mg/(kg·day), and the control group received an equivalent volume of 0.5% CMC-Na solution. The mice had free access to food and water in a temperature-controlled room (23–25 °C) with a 12-h light-dark cycle.

After three weeks of feeding, the body weight of each mouse was recorded, and the fecal samples were quickly frozen in liquid nitrogen and stored at –80 °C. Blood samples were taken from the retro-orbital plexus and stored overnight in a 4 °C freezer to obtain separate serum samples for subsequent testing. The mice were sacrificed by cervical dislocation, and the liver, kidneys, spleen, colon, and cecum were collected. The liver and spleen were weighed, and the contents of the colon and cecum were extracted. All biological samples were frozen in liquid nitrogen and stored at –80 °C for further analysis.

2.6.2 Analysis of biochemical parameters

The activity levels of ABTS, MDA, GSH-Px, SOD, and CAT in serum were measured following the instructions provided with each corresponding kit. The results of ABTS were expressed as a percentage of the control; the MDA levels were reported in nmol/mL, and the other values were presented in U/mL.

2.7 Total DNA extraction, PCR and sequencing of fecal samples

Based on previous studies, total DNA was extracted from

fecal samples via the QIAamp DNA Stool Mini Kit. The quality of the extracted DNA, including integrity, purity, and concentration, was assessed via 1% agarose gel electrophoresis and a NanoDrop spectrophotometer (NanoDrop Technologies Inc., Wilmington, DE, USA). The V3-V4 region of the bacterial 16S rRNA gene was amplified via primer pair 338F (5' - ACTCTACGGGAGGCAGCAG-3') and 806R (5' - GGACTACHVGGGTWTCTAAT-3'), which covers a broad taxonomic range. 16S rRNA gene sequencing of the fecal samples was conducted by Shanghai Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China) via the MiSeq system (Illumina, San Diego, CA, USA).

Raw data analysis was carried out on Majorbio Cloud. QIIME2 (<https://qiime2.org/>) filtered the raw data, and the RDP database helped classify the sequences. The α and β diversity analyses were performed with Mothur (<https://forum.mothur.org/>) and QIIME2, respectively. Genus-level biomarkers across groups were identified via a random forest classification model. Biomarker identification was performed via the linear discriminant analysis (LDA) effect size (LEfSe) to characterize the gut microbiota characteristics specific to the different treatments. ASV-level biomarkers across groups were identified via redundancy analysis (RDA). Spearman's correlation between antioxidant indices and microbiota abundance was analyzed via SPSS (version 19.0, Chicago, IL, USA).

2.8 Statistical analysis

The data were presented as the mean ± standard error of the mean (SEM) and analyzed with GraphPad Prism 8 (GraphPad Software Inc., San Diego, CA, USA). One-way analysis of variance (ANOVA) with Tukey's post hoc test was used to assess statistical differences, and $P < 0.05$ was considered statistically significant.

3 Results

3.1 Component determination and *in vitro* antioxidant activity analysis of SPEE

The results of component determination revealed that SPEE contained flavonoids and polyphenols (0.398 ± 0.018 g/100 g and 0.193 ± 0.002 g/100 g, respectively). The *in vitro* antioxidant activity evaluation demonstrated that both the positive controls Vc and SPEE exhibited concentration-dependent radical-scavenging effects. Specifically, as shown in Fig. 1A and 1C, the scavenging efficacy of SPEE on the ABTS radical and hydroxyl radical improved progressively as the concentration increased from 0 mg/mL to 0.75 mg/mL. At a concentration of 0.75 mg/mL, the scavenging effects of SPEE on the ABTS radical and hydroxyl radical reached 73.02 ± 1.49% and 85.10 ± 0.85%, respectively. Similarly, Fig. 1B and 1D reveal the enhanced scavenging effects of SPEE on DPPH and superoxide anions from 0 mg/mL to 1.0 mg/mL, with concentrations increasing to 1.0 mg/mL, resulting in scavenging rates of 95.03 ± 0.12% and 55.50 ± 0.56%, respectively, at this concentration. These findings underscore the excellent antioxidant capacity of SPEE at the *in vitro* level.

3.2 Antioxidant analysis of SPEE in UVB-irradiated HaCaT cells

To investigate the antioxidant effects of SPEE in cells, UVB irradiation was applied to HaCaT cells to establish an oxidative

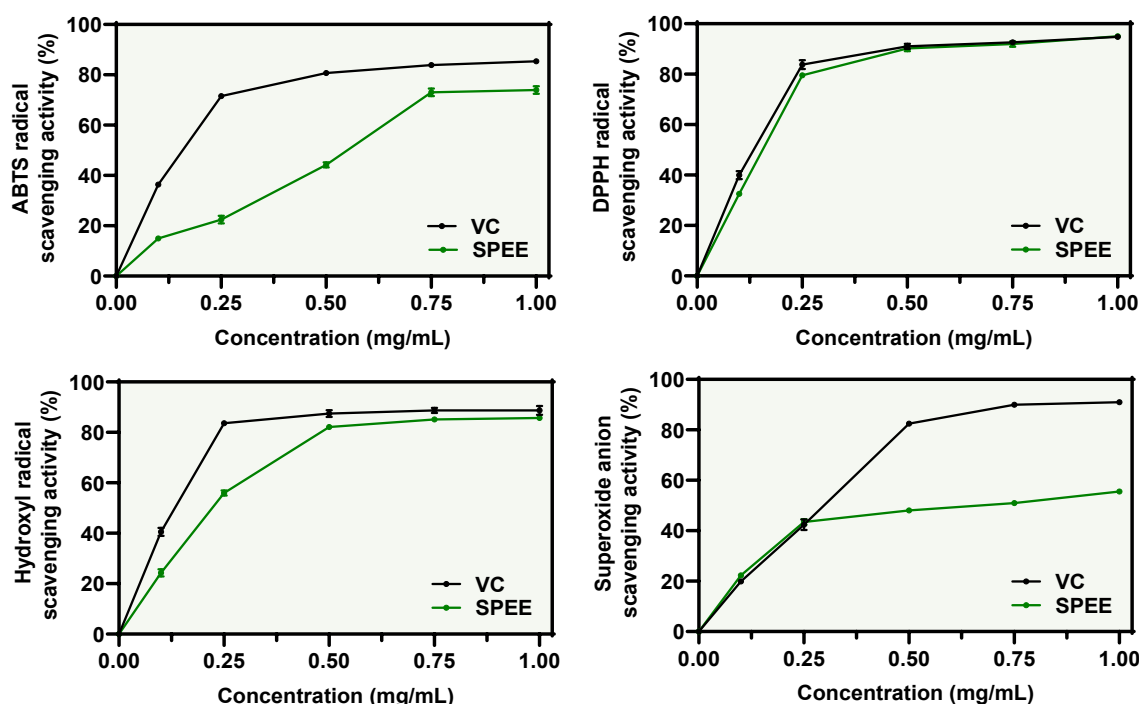


Figure 1 Antioxidant analysis of SPEE *in vitro*. (A) ABTS radical scavenging activity, (B) DPPH radical scavenging activity, (C) Hydroxyl radical scavenging activity, (D) Superoxide anion scavenging activity.

stress model. First, the cytotoxicity of SPEE was detected with a CCK-8 kit. As shown in Fig. 2A, HaCaT cell viability improved as the SPEE concentration increased from 0 $\mu\text{g/mL}$ to 100 $\mu\text{g/mL}$. However, at 200 $\mu\text{g/mL}$, the cell viability decreased to $29.92 \pm 4.10\%$ and further decreased to $8.12 \pm 0.59\%$ at 1000 $\mu\text{g/mL}$. Thus, 0–100 $\mu\text{g/mL}$ was established as the safe concentration range for

SPEE, and concentrations of 25, 50, and 100 $\mu\text{g/mL}$ were employed in the following tests.

To determine the cytotoxicity of different UVB doses and incubation times, HaCaT cells were irradiated with 20 or 40 mJ/cm^2 UVB and then incubated for 2, 8, 12, 16, or 20 h, as illustrated in Fig. 2B. Notably, when the incubation time was 8 h,

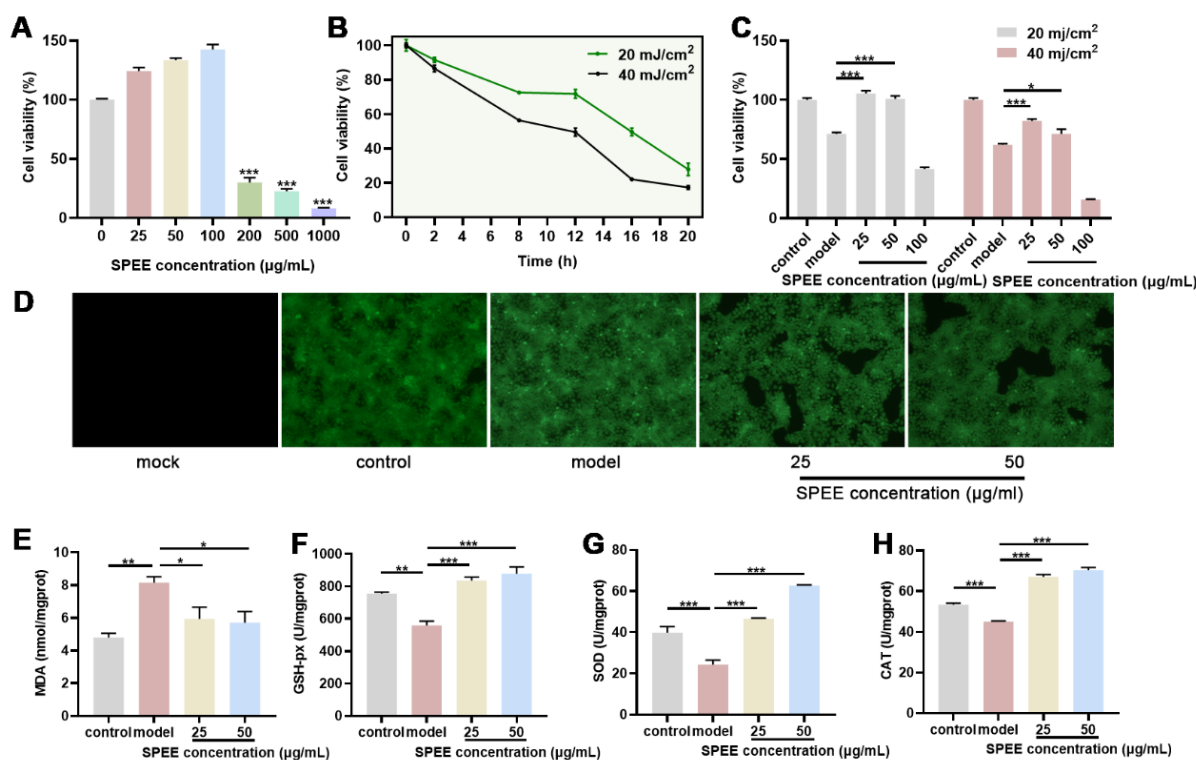


Figure 2 Antioxidant analysis of SPEE in UVB-irradiated HaCaT cells. (A) Effects of SPEE on HaCaT cell viability, (B) Effects of UVB irradiation on HaCaT cell viability, (C) Effect of SPEE on UVB-irradiated HaCaT cell viability, Effects of SPEE on ROS levels (D), MDA (E), GSH-Px (F), SOD (G) and CAT (H) in UVB-irradiated HaCaT cells. Data are represented as mean $\pm$ SEM. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

the cell viability began to decrease to 55%–65% under both UVB doses, so subsequent experiments were conducted under these conditions.

According to the above UVB-irradiated conditions, the effect of SPEE on the viability of UVB-irradiated HaCaT cells was detected. As shown in Fig. 2C, compared with the control conditions, increased SPEE concentrations significantly increased cell viability under 20 and 40 mJ/cm² UVB irradiation ($P < 0.05$). However, when the SPEE concentration increased to 100 µg/mL, the cell viability significantly decreased ($P < 0.05$) compared with that of the model group. In general, 25 and 50 µg/mL SPEE had therapeutic benefits, especially in cells subjected to 20 mJ/cm² UVB, which were chosen for further study.

The effects of SPEE on ROS levels in UVB-irradiated HaCaT cells were observed via fluorescence microscopy, as shown in Fig. 2D. The fluorescence intensity was semi-quantitatively analyzed via ImageJ, and the average fluorescence intensity of the control group was 35.000, whereas that of the model group increased to 52.106. However, after 25 and 50 µg/mL SPEE treatment, the average fluorescence intensity continued to decrease, with values of 43.305 and 37.606, respectively. As shown in Figs. 2E–2H, compared with the control group, the model group presented significantly decreased levels of GSH-Px (559.6 ± 25.67 U/mg prot, $P < 0.01$), SOD (24.31 ± 2.22 U/mg prot, $P < 0.001$) and CAT (45.00 ± 0.40 U/mg prot, $P < 0.001$) but increased levels of MDA (8.17 ± 0.36 nmol/mg prot, $P < 0.01$). However, with the 25 and 50 µg/mL SPEE treatments, the levels of GSH-Px, SOD, and CAT were restored and significantly increased ($P < 0.001$), whereas the levels of MDA were significantly reduced ($P < 0.05$), illustrating the potential of SPEE to mitigate oxidative damage in UVB-irradiated HaCaT cells.

3.3 Antioxidant analysis of SPEE in healthy mice

As displayed in Fig. 3A, there were no significant differences in body weight among the control, LD, and HD groups. Compared with that in the control group, the spleen index (Fig. 3B) significantly increased in the HD group (4.41 ± 0.24 mg/g body weight, $P < 0.01$), whereas the liver index (Fig. 3C) significantly

decreased in the HD group (43.28 ± 1.80 mg/g body weight, $P < 0.05$). Additionally, compared with those in the control group, the serum ABTS radical scavenging activity (Fig. 3D) and the levels of serum GSH-Px (Fig. 3E) significantly increased in the HD group, with values of $33.04 \pm 0.57\%$, $P < 0.01$, and 318.70 ± 35.90 U/mL, $P < 0.05$, respectively, whereas the levels of MDA (Fig. 3F) significantly decreased (8.09 ± 0.90 nmol/mL, $P < 0.01$). However, no significant changes were observed in the levels of SOD (Fig. 3G) and CAT (Fig. 3H) among these three groups.

3.4 The impacts of SPEE on the gut microbiota structure in mice

After three weeks of feeding, 16S rRNA gene sequencing was employed on the fecal samples to explain how SPEE affects the gut microbiota structure. In this study, an average of 28,187 bacterial 16S rDNA reads per sample were obtained. As depicted in Figs. 4A–4D, the Ace and Chao indices decreased in the LD group compared with those in the control group, suggesting a lower richness of the gut microbiota. Furthermore, the Shannon index decreased, and the Simpson index increased in the LD group compared with the control group, indicating lower diversity of the gut microbiota. However, both richness and diversity began to recover in the HD group, although they did not reach the levels of the control group. As shown in Fig. 4E, β -diversity was analyzed via principal coordinate analysis (PCoA), and the pattern of distribution among the three groups followed this order: control, LD, and HD, indicating that SPEE treatment caused shifts at least in part in the gut microbiota structure compared with that of the control group.

Most of the gut microbiota (>89.04%) belonged to *Bacteroidetes* and *Firmicutes* at the phylum level. Compared with the control group, the LD group presented lower abundances of *Bacteroidetes*, *Firmicutes*, *Campilobacterota*, and *Desulfobacterota* but higher levels of *Actinobacteria* and *Patescibacteria*. Compared with those in the control group, the abundances of *Firmicutes* and *Campilobacterota* in the HD group further decreased, *Bacteroidetes* were restored to control levels and even increased,

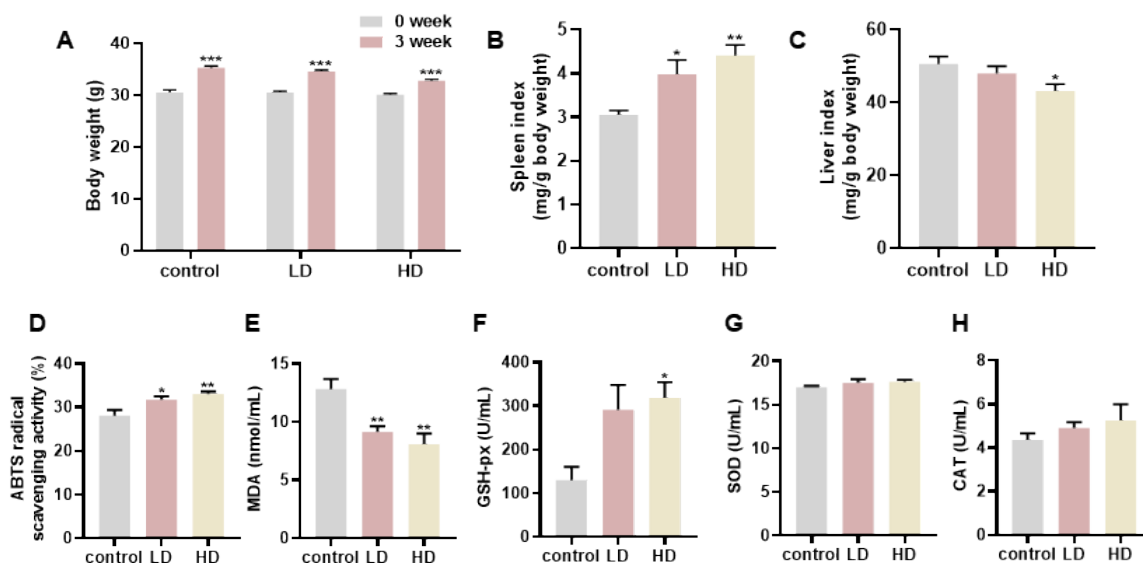


Figure 3 Antioxidant analysis of SPEE in healthy mice. Effects of SPEE on body weight (A), the spleen index (B), and the liver index (C) in healthy mice. Effects of SPEE on ABTS radical scavenging activity (D), MDA (E), GSH-Px (F), SOD (G) and CAT (H) in healthy mice. Data are represented as mean $\pm$ SEM. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

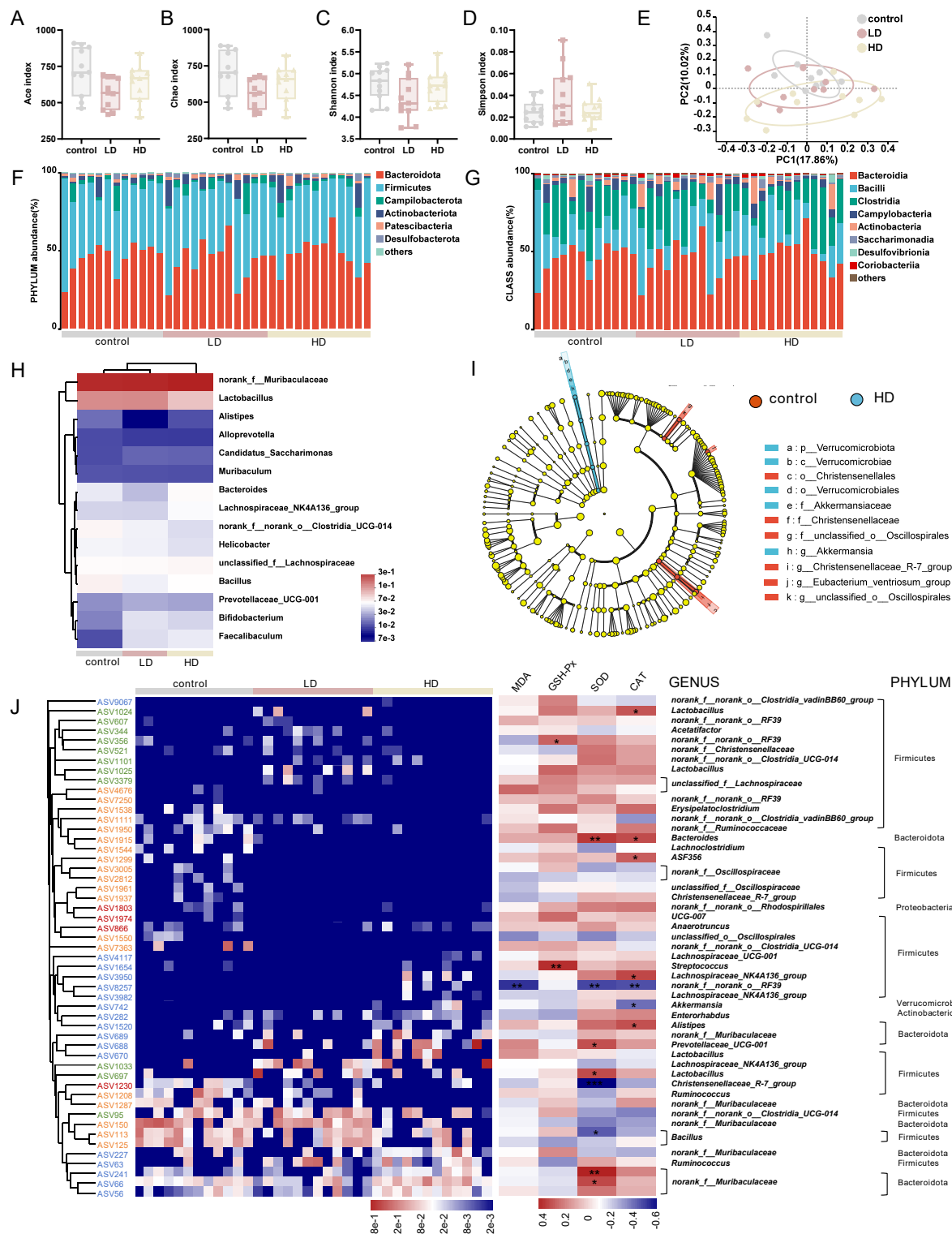


Figure 4 The impacts of SPEE on the gut microbiota structure in mice. Microbial α -diversity was assessed via the Ace index (A), Chao index (B), Simpson index (C), and Shannon index (D), (E) Microbial β -diversity was assessed via PCoA, Bacterial taxonomic profiling at the phylum (F) and class (G) levels, (H) relative abundance of 25 biomarkers at the genus level identified via the random forest classification model, (I) Biomarkers identified via LEfSe in the fecal microbiota. Red, control group; blue, HD group. (J) The relative abundance of 51 key ASVs identified via RDA ($P < 0.05$) and Spearman's correlation analysis between ASV abundance and antioxidant indices. Compared with that in the control group, the abundance of ASVs significantly increased and decreased in the LD group, as indicated with green and red, respectively, and the abundance of ASVs significantly increased and decreased in the HD group compared with the control group, as indicated with blue and orange, respectively. The left heatmap showed the abundance of ASVs, and the right heatmap showed the correlation coefficient values. Data are represented as mean $\pm$ SEM. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

and the abundances of other phyla in the HD group were similar to those in the control group (Fig. 4F and Table S1). At the class

level, the majority of the microbiota consisted of *Bacteroidia*, *Bacilli*, and *Clostridia*, accounting for approximately 88.99% of the

total. Compared with those in the control group, the abundances of *Bacteroidia*, *Clostridia*, *Campylobacteria*, and *Desulfovibrionia* in the LD group were lower, whereas those of *Bacilli*, *Actinobacteria*, *Saccharimonadia*, and *Coriobacteriia* were greater. In the HD group, the abundance of *Bacteroidia* returned to the control level, the abundance of *Bacilli* and *Campylobacteria* were further reduced, and the abundances of other classes remained stable (Fig. 4G and Table S2).

The random forest classification model identified 15 genera as biomarkers for distinguishing the control, LD, and HD groups (importance > 1). The sample clustering tree revealed that the control and LD groups presented greater similarity, whereas the HD group presented less similarity to the other two groups as the SPEE concentration increased. After SPEE treatment, the genera *Alloprevotella*, *Muribaculum*, *Clostridia_UCG-014*, and *Helicobacter* presented continuous downward trends, whereas the genera *Candidatus Saccharimonas*, *Lachnospiraceae_NK4A136_group*, *Lachnospiraceae*, *Bifidobacterium*, and *Faecalibaculum* presented continuous upward trends (Fig. 4H).

LEfSe analysis revealed no significant difference in species among the control, LD, and HD groups, which could be the high similarity between the control and LD groups. However, significant differences in species were observed between the control and HD groups. Among them, the control group was characterized by a greater prevalence of the order *Christensenellales*, the family *Oscillospirales*, the genus *Christensenellaceae_R-7_group*, the family *Christensenellaceae*, and the genus *Eubacterium_ventriosum_group*. Moreover, the HD group presented a greater prevalence of the phylum *Verrucomicrobiota*, the family *Akkermansiaceae*, the class *Verrucomicrobiae*, the order *Verrucomicrobiales*, and the genus *Akkermansia* (Fig. 4I).

Additionally, 51 key ASVs responsive to SPEE treatment were identified via RDA ($P < 0.05$). Among these, the LD group showed significant increases in 11 ASVs and decreases in 4 ASVs compared to the control group. The HD group presented significant increases in 17 ASVs and decreases in 19 ASVs. Spearman's correlation analysis was conducted to correlate these ASVs with MDA, GSH-Px, SOD, and CAT levels in the serum. Among the 51 ASVs, 40 presented positive correlations with antioxidant indices, whereas 11 presented negative correlations. Among these, 15 ASVs were significantly correlated with at least one index ($P < 0.05$), and 2 key ASVs were significantly correlated with at least two physiological indices; these belonged to the *Firmicutes* and *Bacteroidetes* at the phylum level. Notably, SOD was significantly correlated with eight biomarkers, followed by CAT with 7 biomarkers (Fig. 4J and Table S3).

4 Discussion

Although research on the antioxidant properties of *S. pallidum* is abundant, this study systematically evaluated the antioxidant activity of SPEE and analyzed the gut microbiota composition via 16S rRNA gene sequencing, preliminarily revealing potential antioxidant mechanisms that are relatively rare in similar studies.

An increasing number of studies have confirmed the antioxidant activity of natural product-derived extracts or compounds, emphasizing their great potential for use as phytochemical antioxidants against oxidative damage. Recently, *S. pallidum* has attracted the attention of numerous researchers in the biomedical field because of its high bioavailability, abundant phytochemical properties, and strong antioxidant activities^[4,20,21].

S. pallidum is a traditional edible brown alga and herbal medicine renowned for its multiple pharmacological activities. Many studies have revealed a variety of antioxidant compounds from *S. pallidum*, among which flavonoids, polysaccharides, and some simple phenolic compounds are the main bioactive substances^[22]. Moreover, the excellent antioxidant activity of plant extracts may be attributed to the combined synergetic effect of various antioxidants, which is highly correlated with their total flavonoid content (TFC) and total phenolic content (TPC)^[23]. In this study, the major antioxidant components of SPEE were quantified, with a flavonoid content of 0.398 ± 0.018 g/100 g and a total polyphenol content of 0.193 ± 0.002 g/100 g, which were greater than the total phenolic content in the 70% aqueous ethanol extract of *S. pallidum* (3.47 ± 0.19 mg/g)^[11].

ABTS, DPPH, hydroxyl, and superoxide radicals are significant free radicals in the human body, and they can be widely used to measure the ability of different active substances to scavenge free radicals. Flavonoids, phenolics, and other antioxidant compounds in plants donate hydrogen atoms to RO• and ROO• radicals, converting them into stable phenolic radicals, thereby scavenging free radicals and inhibiting peroxidation chain reactions^[24]. In this study, the antioxidant activity of SPEE was progressively validated. First, *in vitro* antioxidant capacity was demonstrated by effectively scavenging the four radicals. This result was similar to that of a previous study, which reported that phenolics and flavonoids from the *Houttuynia cordata* ethyl acetate extract fraction showed good antioxidant activities^[25]. In addition, many studies have shown that polysaccharides from seaweed also have a strong effect on antioxidation. This determination is supported by the findings of Xiao *et al.*, who demonstrated that two sulfated polysaccharides derived from *S. carpophyllum* were rich in sulfate groups and uronic acid, and presented significant antioxidant activity against hydroxyl, superoxide, DPPH, and ABTS radicals. They also reported that the structural characteristics and molecular weights of bioactive compounds affect the hydrogen-donating ability of hydroxyl groups and antioxidant activity^[26]. Thus, the superior antioxidant activity of SPEE is likely attributed to the presence of these bioactive compounds together with their synergistic effects.

UVB exposure leads to apoptotic body formation and DNA damage in severe cases and oxidative stress due to the aggravation of ROS formation^[27]. Previous studies have demonstrated that fucoidan from *S. confusum* reduced UVB-stimulated oxidative stress by blocking the activation of the mitochondrial-mediated apoptosis signaling pathway in HaCaT cells^[28]. UVB can damage mitochondria and disturb the intracellular redox equilibrium, thereby causing excessive ROS generation and reducing the levels of components of the endogenous antioxidant system, such as SOD and GSH-Px^[29]. The results of the present analysis indicate that SPEE exhibited antioxidant properties at the cellular level in UVB-irradiated HaCaT cells, significantly reducing ROS and MDA levels and enhancing the activities of endogenous antioxidant enzymes such as GSH-Px, SOD, and CAT. Gheda *et al.* confirmed that the phenolic extract produced by *Cystoseira compressa* could significantly reduce the lipid peroxidation reaction and the final product MDA of lipid peroxidation caused by excessive ROS^[30], which aligns with our study. Furthermore, *in vivo* tests using mice to further assess the antioxidant activity of SPEE revealed a significant increase in the spleen index, reductions in serum ABTS radical and MDA levels, and an increase in GSH-Px. Collectively, these observations confirmed the strong antioxidant ability of SPEE both *in vitro* and *in vivo*.

The gut microbiota may play critical roles in regulating oxidative stress. On the one hand, certain bacteria in the gut can alter the redox balance by producing oxidases/antioxidant enzymes or mediating the oxidation-related signaling pathway of the host^[31,32]. On the other hand, the diversity of the gut microbiota also regulates the production of ROS. Dysbiosis causes inflammatory cascade activation and ROS overproduction by disrupting the intestinal epithelial barrier and subsequent invasion of opportunistic pathogens^[33–35]. Therefore, the bridge between oxidative stress and the gut microbiota may be changes in gut barrier function. Analysis of the gut microbiota structure revealed that SPEE modulated the gut microbiota, linking its antioxidant mechanism to these microbial changes. After treatment, there was a decrease in the richness and diversity of the mouse gut microbiota, with partial structural changes. At the phylum level, the abundance of *Firmicutes* and *Bacteroidetes* decreased. At the class level, the abundance of *Bacilli* and *Bacteroidia* decreased. At the genus level, the abundance of *Helicobacter* decreased, whereas that of *Bifidobacterium* increased. *Akkermansia* became a biological marker of the treated group. *Helicobacter*, a harmful bacterium, adheres to the gastric mucosa and intercellular spaces, stimulating neutrophils, macrophages, and gastric epithelial cells to release ROS, thereby increasing oxidative stress in the stomach^[36]. Conversely, *Bifidobacterium* exhibits significant antioxidant properties, reducing MDA levels to curb lipid peroxidation, enhancing the bioactivity of SOD in the blood, and hastening free radical clearance^[37]. *Akkermansia* effectively boosts mitochondrial oxidation and bile acid metabolism along the gut-liver axis and alleviates oxidative stress-induced intestinal cell apoptosis^[38]. These findings suggest that SPEE may exert its antioxidant effects by modulating the abundance of the gut microbiota.

In summary, this study systematically demonstrated the *in vitro* and *in vivo* antioxidant activity of SPEE and revealed its modulatory effects on the gut microbiota. This study also preliminarily elucidated the relationship between its antioxidant activity and the gut microbiota. However, the specific mechanisms by which certain antioxidant-related bacteria influence the antioxidant activity of the extract remain unclear and require further investigation.

Electronic Supplementary Material (ESM)

The online version contains supplementary material available at <https://doi.org/10.26599/FMH.2027.9420154>.

Data availability

All data needed to support the conclusions in the paper are presented in the manuscript and/or the Electronic Supplementary Material. Additional data related to this paper may be requested from the corresponding author upon request.

Conflicts of interest

The authors declare that there are no conflicts of interest in this work. Chenyang Lu is an Editorial Board Member of Food & Medicine Homology, but was not involved in the processing, peer review or decision making of this article.

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Author contribution statement

Author 1: Methodology, Software, Validation, Formal Analysis, Data Curation, Writing-Original Draft, Visualization. Author 2: Software, Validation, Formal Analysis, Investigation, Data Curation. Author 3: Software, Validation, Formal Analysis, Visualization. Author 4: Validation, Formal Analysis, Visualization. Author 5: Formal Analysis, Visualization. Author 6: Formal Analysis, Data Curation. Author 7: Conceptualization, Methodology, Writing-Review & Editing, Project Administration. Author 8: Conceptualization, Methodology, Writing-Review & Editing, Supervision, Project Administration, Funding Acquisition. All the authors have approved the final manuscript.

Informed consent

not applicable.

Ethics statement

The animal experiments were carried out following the guidelines of Ethical Committee of Experimental Animal Care at Ningbo University. The protocol was approved by based on the standard guidelines and rules of this institution (NBU12157).

Use of AI statement

None.

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