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Original Research Article

Fermented *Liparis nervosa:* Antioxidant Properties and Its Protective Effects Against Ulcerative Colitis

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Abstract: This study investigated *Liparis nervosa*, an *Orchidaceae* medicinal plant, to systematically optimize its fermentation process using microbial fermentation technology and to evaluate its therapeutic potential against ulcerative colitis (UC). Based on single-factor experiments and orthogonal design, total antioxidant capacity was used as the primary evaluation index, and the optimal fermentation conditions were determined as follows: 1% aqueous extract concentration, fermentation temperature of 35 °C, and fermentation time of 4 days. Under these conditions, the antioxidant activity of the fermented product was significantly enhanced, accompanied by increased flavonoid content and hydroxyl radical scavenging capacity, while total phenolic content decreased, suggesting that fermentation promotes structural transformation and functional optimization of bioactive components. In a dextran sulfate sodium (DSS)-induced mouse model of UC, fermented *Liparis nervosa* extract significantly improved general physiological conditions, reduced the disease activity index (DAI), and alleviated colon shortening and tissue damage. At the molecular level, fermented *Liparis nervosa* downregulated pro-inflammatory cytokines, including *TNF-α, IL-6*, and *NF-κB*, while upregulating intestinal barrier-related proteins such as *ZO-1*, *Claudin-1*, and *Mucin-2*, thereby promoting mucosal repair. In conclusion, optimized fermentation enhances the antioxidant and anti-inflammatory activities of *Liparis nervosa*, enabling it to ameliorate UC through multiple mechanisms, including inflammation suppression, intestinal barrier restoration, and modulation of gut microbiota. This study provides a theoretical and experimental basis for the development of *Liparis nervosa*-based functional foods and traditional Chinese medicinal preparations.

Keyword: *Liparis nervosa*; fermentation; process optimization; ulcerative colitis; anti-inflammatory.

INTRODUCTION

Liparis nervosa (Thunb.) Lindl., a medicinal plant of the Orchidaceae family, is widely distributed in regions such as Jiangxi, Hunan, Fujian, Taiwan, and southwestern China. Traditionally, it is used for cooling blood, hemostasis, and detoxification, and is commonly applied in the treatment of hematemesis, hemoptysis, intestinal bleeding, metrorrhagia, traumatic hemorrhage, abscesses, snake bites, and injuries(L. Zhao *et al.,* 2018). Previous studies have demonstrated that polysaccharides and total alkaloids from *Liparis nervosa* exhibit inhibitory effects on inflammatory factors. Moreover, its ethanol extract significantly shortens bleeding and coagulation time in mice(Ye *et al.,* 2015), suggesting potential therapeutic effects in ulcerative colitis. Pharmacological studies further indicate that *Liparis nervosa* possesses hemostatic, anti-inflammatory, antibacterial, antioxidant, and anticancer activities(J. Li *et al.,* 2025; J. Zhao, Xu, Gao, & Liu, 2023), as well as antihypertensive effects (Chen *et al.,* 2025).

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Fermentation technology, as an ancient biotechnology, has been widely applied in food preservation and flavor enhancement for thousands of years. Through microbial metabolism, fermentation transforms macronutrients such as starch, proteins, and lipids into bioactive metabolites, thereby enhancing both nutritional value and sensory properties. Fermentation is also an essential step in traditional Chinese medicine processing, as recorded in early classical texts(Yan, Feng, Liu, Hu, & Zhang, 2023). Applying fermentation to medicinal and edible plants can generate novel metabolites, enhance efficacy, reduce toxicity, and improve palatability (Y. Li *et al.,* 2026).

Ulcerative colitis (UC) is a chronic inflammatory disease affecting the colon and gastrointestinal tract. Its incidence has been increasing globally and is associated with genetic susceptibility, epithelial barrier dysfunction, immune dysregulation, and environmental factors (Head & Jurenka, 2003; Rubin, Ananthakrishnan, Siegel, Barnes, & Long, 2025; Sun *et al.,* 2025). Conventional treatments, including 5-aminosalicylic acid and corticosteroids, often present limitations such as long treatment duration and adverse effects, leading to poor patient compliance (Chaemsupaphan, Arzivian, & Leong, 2025; Ferretti, Cannatelli, Monico, Maconi, & Ardizzone, 2022; R. Li & Yang, 2025). Recently, traditional Chinese medicine has shown promising therapeutic potential in UC management (Y. Huang, Ma, Xu, & Liu, 2025; Zheng, Xue, Wang, Guo, & Liu, 2022).

Therefore, this study aims to optimize the fermentation process of *Liparis nervosa* and investigate its therapeutic effects in a DSS-induced UC mouse model by analyzing inflammatory cytokines and intestinal barrier proteins. The findings are expected to provide theoretical support for its application in UC prevention and treatment, as well as for the development of functional foods and medicinal products.

MATERIALS AND METHODS

Fermentation of *Liparis nervosa*

Fresh *Liparis nervosa* was washed, boiled in distilled water for 10 min, filtered, and sterilized. After cooling below 35 °C, different proportions of *Pichia pastoris* were added for fermentation under shaking conditions. All procedures were conducted under sterile conditions.

Preparation of *Pichia pastoris* Suspension

The strain was stored at −20 °C. The stored strain was thawed, and the *Pichia pastoris* cells were inoculated into YPD medium and cultured at 37 °C for 18–24 hours. Determine the concentration of the bacterial suspension by counting the colonies on agar plates, and dilute the suspension to approximately 1×10^6 CFU/ml. Transfer 1 mL of the diluted bacterial suspension to a sterile 1.5 mL centrifuge tube, centrifuge at 3000 rpm for 5 minutes, collect the bacterial cells, add 1 mL of sterile water, and mix thoroughly. Repeat this step once, then add 1 mL of saline and mix thoroughly to prepare a bacterial suspension for subsequent use.

Determination of Bioactive Components

The total antioxidant capacity, flavonoid content, and total phenolic content of fermented *Liparis nervosa* were determined using a kit from Solabio (Beijing) Co., Ltd. (X. Lu *et al.,* 2023; Quan, Li, Liang, Fu, & Wan, 2021; Yang *et al.,* 2023).

The Effect of Different Concentrations of *Liparis nervosa* Water Extract on Total Antioxidant Activity

Preparation of 1–5% *Liparis nervosa* aqueous extracts: Add 1–5 g of *Liparis nervosa* to 100 mL of distilled water in a 250 mL beaker and heat on an electric hotplate. Once the solution comes to a boil, continue heating for 10 minutes. Remove from heat and allow the solution to cool before use. Transfer 5–60 μL of the boiled and sterilized *Liparis nervosa* water extract to each ampoule, then add 240 μL of 1×10^6 CFU/mL *Pichia pastoris*. Incubate in a shaking incubator at 35°C for 4 days. After removal, centrifuge for 4 minutes at 5,000 rpm, then measure the total antioxidant value at 593 nm. Perform three replicates for each concentration gradient.

The Effect of Different Concentrations of *Pichia pastoris* on Total Antioxidant Value

Volumes of 5,880 μL, 5,820 μL, 5,760 μL, 5,700 μL, and 5,640 μL of the *Liparis nervosa* water extract at the optimal concentration were transferred to ampoules. To each, 120 μL, 180 μL, 240 μL, 300 μL, and 360 μL of 1×10^6 CFU/mL *Pichia pastoris*, respectively. The vials were incubated on a shaking incubator at 35 °C for 4 days. After incubation, the samples were centrifuged at 5000 r/min for 4 minutes, and the total antioxidant activity was measured at 593 nm; each concentration gradient was tested three times.

The Effect of Different Fermentation Temperatures on Total Antioxidant Value

Solutions containing the optimal concentrations of *Liparis nervosa* water extract and *Pichia pastoris* were prepared and placed in ampoules. The ampoules were incubated on a shaking incubator at 25 °C, 30 °C, 35 °C, and 40 °C for 4 days. After removal, centrifuge for 4 minutes at 5,000 rpm, then measure the total antioxidant activity at 593 nm. Perform three replicates for each concentration gradient.

The Effect of Different Fermentation Times on Total Antioxidant Value

Solutions containing the optimal concentrations of *Liparis nervosa* water extract and *Pichia pastoris* were prepared and placed in ampoules. These were incubated on a shaking incubator at the optimal fermentation temperature for 2, 3, 4, 5, and 6 days, respectively. After removal, centrifuge for 4 minutes at 5,000 rpm, then

measure the total antioxidant activity at 593 nm; repeat this measurement three times for each concentration gradient.

Optimization of Fermentation Conditions for *Liparis nervosa*

Based on the results of single-factor experiments on the fermentation of *Liparis nervosa*, an orthogonal experiment was designed to optimize the fermentation process conditions. An $L_9(3^4)$ orthogonal design was employed, with three factors selected for the experiment: concentration of *Liparis nervosa* water extract (A), treatment temperature (B), and treatment time (C). Total antioxidant capacity was used as the primary indicator to determine the optimal fermentation conditions. The levels of each factor are shown in Table 1.

Table 1: Table of Factor Levels for $L_9(3^4)$

Level	Factors		
	A Concentration (%) of *Liparis nervosa* aqueous extract	B Processing temperature (℃)	C Processing time (d)
1	1	25	2
2	2	30	3
3	3	35	4

Animal Experiment Design

After 18 experimental mice had been acclimatized for 7 days, they were randomly divided into a control group (6 mice), a model group (6 mice), and a *Liparis nervosa* group (6 mice). Mice in the *Liparis nervosa* group were administered *Liparis nervosa* via gavage for 5 days, while mice in the control and model groups were administered distilled water via gavage during the same period. After 5 days, a DSS-induced model of ulcerative colitis was established in the model group and *Liparis nervosa* group mice according to Reference (M. Wang *et al.,* 2024). The animal experimentation protocol for this study has been reviewed and approved by the Animal Ethics Committee of the Sichuan Academy of Chinese Medicine Sciences. The review resolution number is DWSYLL-2026-009. Mice in the *Liparis nervosa* group were administered a 1% aqueous extract of *Liparis nervosa* via gavage daily, with a volume of 1 mL, for a 5-day period; mice in the other two groups were administered the same volume of distilled water via gavage.

General observations and DAI score

DAI scores were assigned to mice daily based on standards established in the literature. DAI = (weight loss score + stool consistency + occult blood) / 3. The weight loss score was graded on a 5-point scale: 0 points—no weight loss; 1 point—1–5% weight loss; 2 points—5–10% weight loss; 3 points—10–15% weight loss; 4 points—greater than 15% weight loss (Hamamoto *et al.,* 1999). Normal stool consistency is scored as 1-point, semi-loose stools as 3 points, and loose stools as 5 points; negative fecal occult blood is scored as 1-point, positive fecal occult blood as 3 points, and gross fecal blood as 5 points. See Table 2 for the DAI scoring table.

Table 2 DAI scoring table

Score	Weight loss rate	Stool consistency	Hematochezia
1	0	Normal	negative
2	1～5	——	——
3	5～10	Semiliquid stool	Fecal occult blood
4	10～15	——	——
5	＞15	Loose stool	Grossly bloody stool

Note: Normal mouse feces are dry and small, resembling pellets; semi-loose stools are paste-like but do not stick to the anus; and loose stools are liquid and stick to the anus.

Sample collection

Blood samples were first collected from the mice via eye puncture, and the mice were then euthanized by cervical dislocation. The mice were dissected, and their gross morphology was examined. Following dissection, samples were collected from the liver, spleen, kidneys, colon, and cecum, and the contents of the colon and cecum were also collected. The samples were placed in separate 1.5 mL Eppendorf tubes and stored at −80°C (samples from the colon intended for PCR were stored in RNAse-free Eppendorf tubes).

Measurement of colon tissue length

After opening the mouse's abdominal cavity, trim the lower end of the colon near the anus and cut off the upper portion of the cecum along with the cecum itself. This is done to measure the length of the colonic tissue. The length of the colon is measured starting from the lower end of the cecum.

Calculation of Organ Indices

After dissecting the mice, the spleens and kidneys were removed, their weights were recorded, and

the organ indices for the spleen and kidneys were calculated using the measured data. Organ index = (weight of the spleen (or kidney), g) / (mouse body weight, g) × 100

Extraction of total RNA and QRT-PCR

Total RNA from mouse liver was extracted using the Total RNA Kit I (Omega, USA). Reverse transcription to cDNA was performed using HiScript III RT SuperMix for qPCR (+gDNA wiper) (Vazyme, China). β-actin served as the internal control for qRT-PCR reactions using SYBR Green I-based fluorescent detection.(Zhen *et al.,* 2022)

Statistical analysis

All test data are displayed as the mean ± standard deviation (SD) of three repetitions of each experiment. GraphPad Prism 10 was used to analyze statistical differences via one-way ANOVA and Student's t-test. * indicates $P<0.05$, ** indicates $P<0.01$, *** indicates $P<0.001$, and NS indicates no statistical difference.

RESULTS

The concentration of the *Liparis nervosa* aqueous extract has a significant effect on the total antioxidant value, which decreases as the concentration of the extract increases. When the concentration of the *Liparis nervosa* aqueous extract was 1%, the total antioxidant value reached 1.402 μmol/ml, whereas at a concentration of 3%, the total antioxidant value was only 0.853 μmol/ml, representing a significant decrease compared to the 1% concentration. When the concentration of the *Liparis nervosa* aqueous extract was <5%, the total antioxidant value data were favorable, and the fermentation results were excellent; however, the error in the antioxidant value data corresponding to the 3% and 5% concentrations was greater (Fig. 1A). The final selected concentration of the *Liparis nervosa* aqueous extract was 1%. As the concentration of *Pichia pastoris* increased, the total antioxidant value dropped sharply, then rose slightly before continuing to decline. As shown in Figure 1B, the effect of *Pichia pastoris* concentration on the total antioxidant value was relatively modest. When the *Pichia pastoris* concentration was 2%, the total antioxidant value of the solution reached a maximum of 1.431 μmol/ml, which was nearly identical to the minimum value of 1.338 μmol/ml observed at a 3% concentration. The final selected concentration of Saccharomyces cerevisiae was 2%. It is evident that the fermentation temperature has a significant impact on the antioxidant value of the *Liparis nervosa* water extract; the higher the fermentation temperature, the more pronounced the downward trend in the antioxidant value. The peak value of 1.451 μmol/ml was reached at 30°C. Furthermore, the antioxidant values of the *Liparis nervosa* water extracts treated at 25°C, 30°C, and 35°C showed little difference; after a brief increase, they continued to decline (Fig. 1C). Therefore, a fermentation temperature of 30°C was selected.

Fermentation time had a significant effect on the antioxidant activity of the *Liparis nervosa* aqueous extract (Fig. 1D); the antioxidant activity of the extract decreased as fermentation time increased. At a fermentation time of 6 days, the antioxidant value of the *Liparis nervosa* water extract was only 0.979 μmol/ml, whereas at a fermentation time of 2 days, it reached 1.573 μmol/ml, with a significant difference between the two groups. In conclusion, a fermentation time of 2 days was selected.

Using total antioxidant value as the indicator, a three-factor, three-level $L_9(3^4)$ orthogonal experiment was conducted using the three factors of *Liparis nervosa* water extract concentration (A), fermentation temperature (B), and fermentation time (C) to determine the optimal fermentation conditions for *Liparis nervosa*. The results of the orthogonal experiment and analysis of variance are shown in Tables 3 and 4.

As shown in Tables 3 and 4, during the fermentation optimization of *Liparis nervosa*, the order of significance of the three experimental factors on the antioxidant value was: aqueous extract concentration > fermentation time > fermentation temperature. Furthermore, based on the range analysis, the optimal combination of process conditions was determined to be A1B3C3, a 1% concentration of *Liparis nervosa* water extract, a fermentation temperature of 35°C, and a fermentation duration of 2 days. Validation experiments were then conducted using these process conditions for *Liparis nervosa* fermentation, with three parallel replicates. The results showed that the average total antioxidant value of the *Liparis nervosa* aqueous extract was 1.792 μmol/ml, confirming that the selected optimal process condition combination is reasonable.

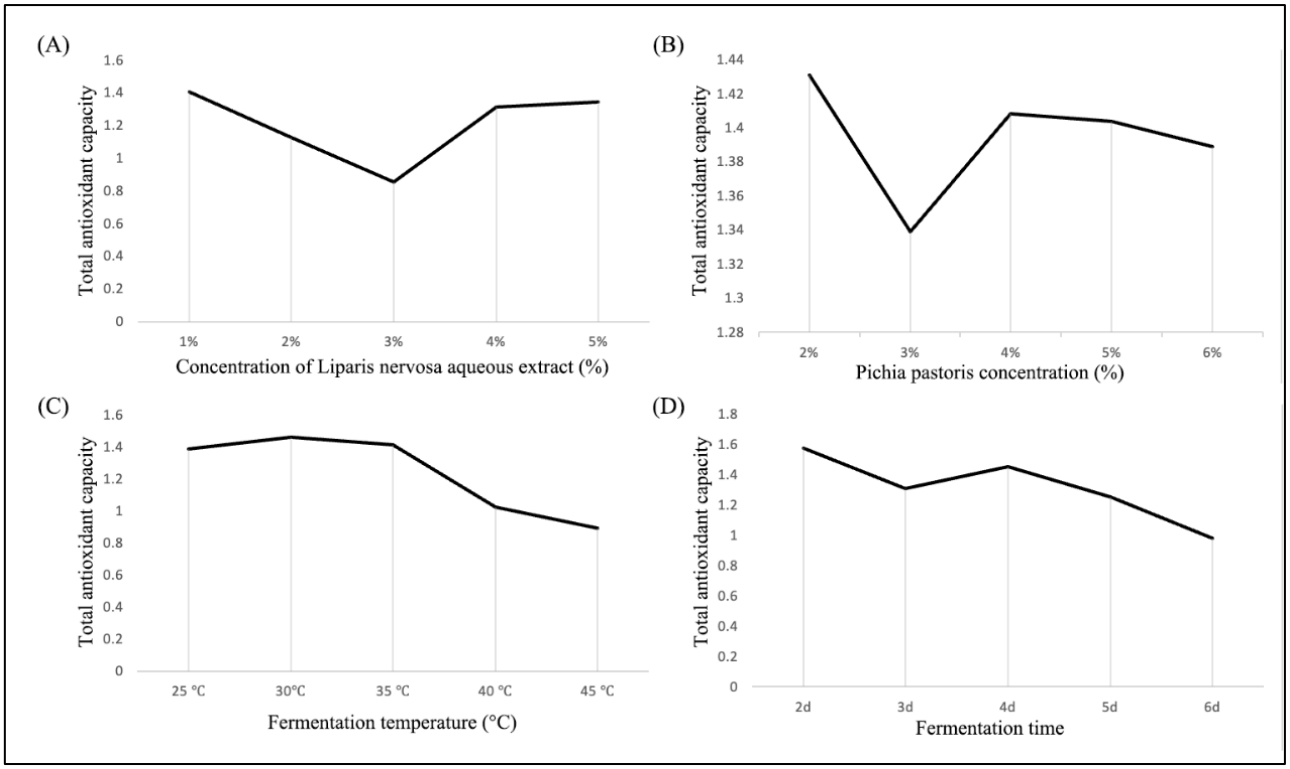

Fig.1 The Effect of Different Treatment Methods on the Total Antioxidant Capacity of *Liparis nervosa*

Table 3 Optimization Protocol and Analysis of Fermentation Results for *Liparis nervosa*

Test Number	Factors			Total Antioxidant Value (μmol/ml)
	A (g/L)	B (℃)	C (min)	
1	1	1	1	0.788
2	1	2	2	1.013
3	1	3	3	1.809
4	2	1	2	0.935
5	2	2	3	1.092
6	2	3	1	0.757
7	3	1	3	1.641
8	3	2	1	1.114
9	3	3	2	1.434
K1	1.203	0.928	1.396	
K2	1.121	1.073	1.127	
K3	0.886	1.333	1.514	
R	0.317	0.405	0.387	

Table 4: Analysis of Variance for the Fermentation Optimization Experiment of *Liparis nervosa*

Sources of variance	Sum of squares	Degrees of freedom	F-ratio	Significance
A	0.332	2	5.922	**
B	0.115	2	2.050	*
C	0.602	2	20.718	*
Error	0.056	2		
Total	13.550	9		

Significant changes were observed in total phenolic content, flavonoid content, and hydroxyl radical scavenging capacity. Compared to the original juice, the optimized fermentation process resulted in a significant decrease in total phenolic content and flavonoid content, while the hydroxyl radical scavenging capacity increased significantly. Based on the analysis results of the above single-factor and orthogonal experiments, and in conjunction with the comparison of data on various components in the *Liparis nervosa* water extract before and after fermentation presented in Table 5, the results indicate that Group 7 exhibited the highest antioxidant value. The optimal fermentation process conditions were determined to be: a concentration of 1% *Liparis nervosa*

water extract, a shaking incubator temperature of 35°C, and a fermentation duration of 4 days.

Table 5: Comparison of Various Components in *Liparis nervosa* Before and After Treatment Under Different Fermentation Conditions

	Liparis nervosa water extract	**After fermentation optimization**
Flavonoids（mg/g）	0.0783	0.114
Total phenolic content（mg/g）	0.0626	0.0432
Hydroxyl radical scavenging activity（%）	2	12

General observations and DAI scores showed that mice in the control group had shiny coats, were active, and exhibited normal levels of activity and food intake. Mice in the model group had dull, lackluster coats, appeared lethargic, were less active, and showed reduced food and water intake, as well as loose stools; in contrast, mice in the *Liparis nervosa* group exhibited alleviated symptoms. Compared with the model group, mice in the *Liparis nervosa* group regained their appetite, were more active, and showed increased physical activity. Compared with the control group, the DAI score in the model group was significantly higher than that in the control group, with the difference reaching statistical significance ($P \leq 0.05$); compared with the model group, the DAI score in the *Liparis nervosa* group was lower, and the difference was also statistically significant ($P \leq 0.05$); However, the difference between the *Liparis nervosa* group and the control group was not statistically significant ($P > 0.05$) (Table 6). These results indicate that *Liparis nervosa* can effectively alleviate weight loss in mice caused by ulcerative colitis, suggesting that *Liparis nervosa* may have a certain protective effect against ulcerative colitis.

Table 6 DAI scores of each group ($\bar{x} \pm s$)

Group	**n**	**DAI scores**
CG	6	0.59±0.15△
MG	6	2.67±0.71
JG	6	2.01±0.47△

Note: △ indicates a $P \leq 0.05$ compared to the model group.

Colon length is one of the key indicators used to assess the severity of ulcerative colitis. Specifically, a shorter colon length indicates more severe inflammation, whereas a longer colon length indicates milder inflammation. Upon macroscopic examination, the colonic mucosa of the control group mice was smooth with no adhesions, and no ulcers were observed. In contrast, the colons of mice in the model group exhibited varying degrees of shortening, accompanied by mild congestion and edema. In contrast, the colons of mice in the *Liparis nervosa* group showed no obvious tissue edema or congestion, and their colon lengths fell between those of the control and model groups (Fig. 2). Based on these morphological changes in the colons, we conclude that *Liparis nervosa* exerts a preventive effect against ulcerative colitis in mice.

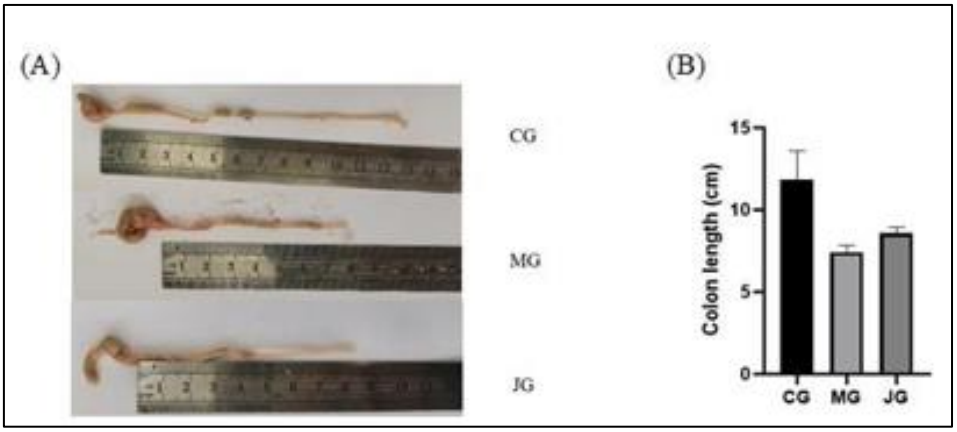

Fig.2 Changes in colon tissue length in mice

At the same time, compared with the control group, the organ indices of mice in the model group were elevated (Table 7). Meanwhile, compared with the model group, the organ indices of mice in the *Liparis nervosa* group were reduced. This indicates that after drinking the DSS solution, the internal organs of the mice were damaged by the toxin, resulting in elevated organ indices. Thus, it can be concluded that after consuming

the DSS solution, the mice's internal organs were affected by the toxin, and this experiment successfully established a mouse model of ulcerative colitis.

Table 7: Organ Index for each group

Group	n	Organ Index
CG	6	1.29±0.051
MG	6	1.54±0.079
JG	6	1.34±0.053

According to the PCR results, the expression levels of *TNF-α, IL-6*, and *NF-κB* in the colon tissue of mice in the model group were significantly higher than those in the control group (Fig. 3). Compared with the model group, the expression levels of *TNF-α, IL-6*, and *NF-κB* in the colon tissues of mice in the *Liparis nervosa* group were reduced, indicating that *Liparis nervosa* can, to some extent, reduce the inflammatory response in the mouse colon. This indicates that the experimental results are consistent with expectations.

Compared with the control group, the expression of *ZO-1* (a tight junction protein) in the colon tissue of mice in the model group was significantly reduced. In contrast, compared with the model group, the expression of *ZO-1* protein in the colon tissue of mice in the *Liparis nervosa* group was increased (Fig. 4). Additionally, after successful model establishment, the mRNA expression of *Mucin-2* in the colon tissue of the model group mice decreased compared to the blank group, while the expression of *Mucin-2* in the colon tissue of the *Liparis nervosa* group showed an upward trend. Furthermore, compared to the blank group, the expression of *Claudin-1* (tight junction protein) in the colon tissue of the model group mice was significantly reduced. However, compared to the control group, *Claudin-1* expression was increased in the *Liparis nervosa* group. Therefore, measurements of mucin and tight junction protein levels in mouse colon tissue revealed that *Liparis nervosa* promotes the secretion of both proteins, thereby facilitating the repair of the mouse colon mucosa.

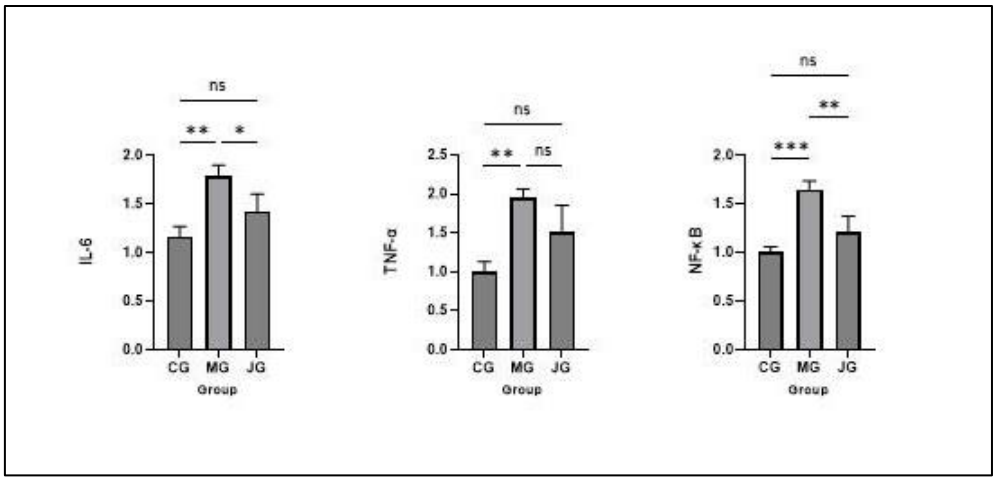

Fig.3 The effect of Liparis nervosa on IL-6、TNF-α、NF-κB expression in each group of mice was seen

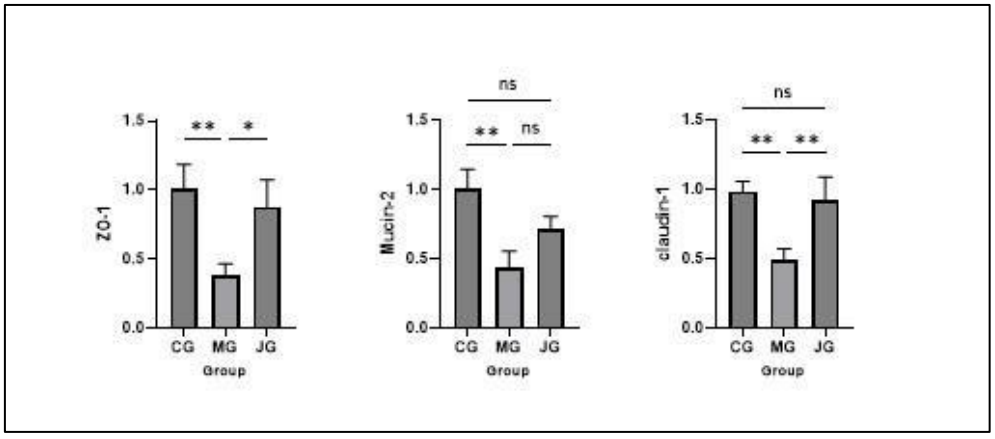

Fig.4: ZO-1, Mucin-2, Claudin-1 mRNA expression

DISCUSSION

Current research on *Liparis nervosa* continues to focus primarily on the isolation of chemical constituents and the evaluation of pharmacological activities. Reported classes of compounds include *neuronic acid derivatives, phenanthrenes, biphenanthrenes, pyrrolizidine alkaloids*, and *phenylpropanoids* (J. Zhao *et al.,* 2023); pharmacological studies have mainly focused on hemostatic, procoagulant, anti-inflammatory, antibacterial, antitumor, and antioxidant effects(J. Li *et al.,* 2025; L Liu *et al.,* 2024). Plants of the genus Liparis have long been used in China for the treatment of traumatic bleeding, inflammation, and detoxification, and their representative active components are precisely the aforementioned diverse secondary metabolites (Liang, Guo, Nagle, Zhang, & Tian, 2019).

Given this research background, the significance of this study lies not in re-establishing that *Liparis nervosa* "possesses bioactivity," but rather in determining whether fermentation can reorganize the state of its active compounds and further enhance its antioxidant performance. This aligns well with existing literature. Previous studies have shown that certain phenylpropanoid compounds isolated from *Liparis nervosa* exhibit strong in vitro antioxidant activity, with some individual compounds even outperforming vitamin C in DPPH and ABTS assays; furthermore, the essential oil of *Liparis nervosa* has also been reported to possess in vitro antioxidant activity (Liang Liu, Zou, Yin, Zhang, & Zhang, 2021). In other words, *Liparis nervosa* inherently possesses the material basis for antioxidant effects; therefore, the key to fermentation optimization is not "generating bioactivity from scratch," but rather promoting the release, transformation, or enrichment of pre-existing active components.

In this study, as the concentration of the *Liparis nervosa* water extract increased, the total antioxidant value actually decreased, with the best performance observed at a 1% concentration. This result indicates that, in this fermentation system, higher substrate concentrations do not necessarily yield better results. Considering the general principles of traditional Chinese medicine fermentation, higher extract concentrations often imply higher viscosity, a more complex chemical environment, and a greater risk of metabolic inhibition; although microorganisms are exposed to more substrate, they may not necessarily complete biotransformation more efficiently. Conversely, a lower concentration of the aqueous extract is more conducive to maintaining stable growth of *Pichia pastoris*, allowing the microorganisms to focus their metabolic capacity on the conversion of active ingredients rather than on environmental adaptation. Therefore, the 1% concentration identified in this study is more likely to correspond to a "window" where the microorganisms can tolerate the conditions, achieve high conversion efficiency, and maintain a relative balance between the production and consumption of active compounds, rather than simply reflecting a dilution effect.

Regarding inoculum size, the 2% *Pichia pastoris* inoculum yielded the highest total antioxidant value, but the difference from adjacent concentrations was not particularly significant. This suggests that the influence of this factor on the system is more "threshold-dependent" rather than "linearly increasing." Existing yeast fermentation studies generally agree that an inoculum density that is too low leads to slow initiation and insufficient metabolic activation, while an inoculum density that is too high accelerates nutrient consumption and causes metabolic competition, ultimately hindering the accumulation of the target product(M. Wang *et al.,* 2019). In the context of this study, this implies that *Pichia pastoris* acts more as a "biotransformation trigger": once the cell density reaches a certain level, it can drive the biotransformation of certain components in *Liparis nervosa*; however, further increases in cell density do not proportionally increase antioxidant activity.

The results for temperature and time also reflect this characteristic of "dynamic equilibrium." In single-factor experiments, 30°C and 2 days yielded relatively high total antioxidant capacity (T-AOC) values; however, after orthogonal optimization, the optimal combination was found to be 35°C and 4 days, with T-AOC reaching 1.809 μmol/mL in the third group. This phenomenon indicates that the fermentation of *Liparis nervosa* is not a simple process dominated by a single factor, but rather a synergistic conversion process jointly determined by substrate concentration, temperature, and time. Under single-factor conditions, shorter durations were more conducive to preserving the original antioxidant substances; however, when multiple factors were combined, the conditions of 35°C and 4 days may have facilitated more thorough enzymatic conversion by the yeast, transforming some of the original components into forms with higher activity and greater reaction efficiency, thereby further increasing the overall antioxidant level. In the orthogonal experiment, the A1B3C3 combination performed best, and range analysis indicated that the order of influence was water extract concentration > treatment time > treatment temperature, further confirming that substrate conditions remain the primary factor determining the direction of fermentation.

More notably, following fermentation, the flavonoid content of *Liparis nervosa* increased, total phenolic content decreased, and hydroxyl radical scavenging activity significantly improved. These results indicate that the enhanced antioxidant activity observed in this study does not follow a simple correlation of "higher total phenolic content equating to stronger activity," but rather likely reflects an optimization of the structural composition and form of the active compounds. Previous studies have shown that *Liparis*

nervosa contains not only neuronic acid derivatives and alkaloids, but also phenylpropanoids and other phenolic compounds with antioxidant potential(S. Huang *et al.,* 2013). During fermentation, microorganisms may promote the consumption or rearrangement of some bound phenols through deglycosylation, ester bond hydrolysis, redox reactions, or fragmentation into smaller molecules, while simultaneously releasing flavonoid-like or phenylpropanoid-like components with higher antioxidant efficiency (Alharbi, 2026; Xie, Shawky, Selim, & Gao, 2026). Therefore, a decrease in total phenolic content does not necessarily imply a reduction in the system's antioxidant capacity; on the contrary, if the decrease involves macromolecules or bound phenols with lower reactivity, while the increase involves small-molecule active components that are more effective at scavenging free radicals, the overall T-AOC and hydroxyl radical scavenging capacity may well increase. In the experiment, flavonoids increased from 0.0783 mg/g to 0.114 mg/g, total phenols decreased from 0.0626 mg/g to 0.0432 mg/g, and the hydroxyl radical scavenging rate increased from 2% to 12%, which precisely aligns with this transformation characteristic of "decreased total content but enhanced activity."

This explanation is also consistent with the current pharmacological research direction of *Liparis nervosa*. Recent studies have shown that *Liparis nervosa* extracts possess distinct anti-inflammatory and neuroinflammation-inhibiting activities, with these effects related to the regulation of NF-κB and MAPK signaling pathways; simultaneously, certain chemical components also exhibit antibacterial, immunosuppressive, and cytotoxic activities(Jiang *et al.,* 2024; L. Li *et al.,* 2025). This indicates that the pharmacological effects of *Liparis nervosa* do not depend on a single component but are based on the synergistic action of multiple classes of bioactive compounds. For this reason, the significance of fermentation likely lies not in a mechanical increase in the content of a single component, but rather in the reshaping of the proportional relationships and structural configurations of various active substances. From this perspective, the antioxidant improvements observed in this study may merely be an external manifestation of the enhanced comprehensive biological activity following *Liparis nervosa* fermentation; subsequent studies can fully verify whether anti-inflammatory or hemostatic-related indicators are simultaneously enhanced.

Furthermore, previous studies have indicated that *Liparis nervosa* possesses distinct hemostatic and procoagulant activities; the n-BuOH fraction can promote ADP-induced platelet aggregation, and *Liparis nervosa* as a whole is considered to have significant hemostatic, procoagulant, and anti-inflammatory potential (Song *et al.,* 2013). This study suggests that the development value of *Liparis nervosa* fermentation products extends beyond their use as antioxidant beverages or functional extracts; it may also expand into fields such as wound healing, inflammation regulation, and functional food ingredients, providing significant reference value for related research. Current experiments have demonstrated that fermentation can improve certain antioxidant parameters, laying the foundation for future efforts to correlate changes in chemical activity with pharmacological effects.

Ulcerative colitis (UC) is a chronic inflammatory disease that affects the colon and other parts of the gastrointestinal tract. The inflammation primarily occurs in the mucosa of the colon and rectum. It is characterized by a prolonged course, recurrent flare-ups, and affects patients across all age groups. In recent years, with China's continuous economic development and rising living standards, the prevalence of UC in China has shown a gradual upward trend. Consequently, clinicians have adopted various treatment regimens for UC, such as corticosteroids, 5-aminosalicylic acid derivatives, biologics, and immunosuppressants. However, the use of these medications can also lead to certain adverse effects; for example, corticosteroids may cause side effects such as obesity(Piodi, Poloni, & Ulivieri, 2014) and osteoporosis. Several biologics and immunosuppressants have been approved in China for the treatment of ulcerative colitis(Miyoshi *et al.,* 2018; K. Wang, Zhu, Liu, Zhu, & Ouyang, 2024). It should also be noted that achieving disease control in ulcerative colitis requires the development of personalized treatment regimens.

Liparis nervosa is a traditional Chinese herbal medicine with anti-inflammatory and hemostatic properties. It is widely distributed, readily available, and cost-effective. Previous studies have demonstrated the anti-inflammatory effects of *Liparis nervosa* (Jiang *et al.,* 2024), leading to the hypothesis that it may have preventive and therapeutic effects on ulcerative colitis. This study found that, compared to the control group, mice in the model group first exhibited softening of stool consistency, leading to loose stools, shortly after the model was established. By the later stages of the experiment, the mice displayed symptoms such as dull fur, weight loss, and loss of appetite, accompanied by elevated DAI scores. In contrast, the *Liparis nervosa* group exhibited a partial alleviation of the UC-induced symptoms compared to the model group. Specifically, the mice's general condition improved, they regained some vitality, and their DAI scores decreased. Clinically, the primary principles of treating ulcerative colitis involve alleviating inflammatory symptoms, reducing the expression of inflammatory factors, and promoting the healing of the intestinal mucosa. Post-mortem examination revealed that the colons of mice in the model group exhibited varying degrees of shortening, accompanied by mild congestion and edema. This contrasted markedly with the colon tissues of mice in the control and *Liparis nervosa* groups. This study demonstrates that *Liparis nervosa* exerts a significant

alleviating effect on DSS-induced ulcerative colitis in mice.

TNF-α is a typical cytokine produced in response to inflammatory reactions. When the expression of pro-inflammatory cytokines in the intestinal tract becomes imbalanced—specifically, when they are overexpressed—it leads to intestinal inflammation and damage; in severe cases, it can result in intestinal cell death (W. Huang *et al.,* 2026). Compared to the control group, the expression levels of the inflammatory factor *IL-6*、*TNF-α*、*NF-κB* were significantly elevated in the colon tissues of mice in the model group; in contrast, expression levels in the colon tissues of mice in the *Liparis nervosa* group were reduced compared to the model group. Therefore, we found that *Liparis nervosa* can control the onset of inflammation by regulating the expression of pro-inflammatory factors.

Studies have shown that when intercellular continuity and integrity are disrupted, inflammatory cells and pathogens can pass through the epithelium unimpeded via the gaps (W. Huang *et al.,* 2026). Under various pathological conditions, proteins with different functional properties that act on the intercellular junctions can ultimately affect intestinal barrier function, epithelial integrity, and the repair of intestinal tissue(Suzuki, 2020). Concurrently, inflammation-induced defects in intestinal barrier function allow bacteria and their antigens to migrate into the underlying intestinal tissues, leading to the persistence of inflammation(Guarino, Di Ciaula, Portincasa, & De Giorgio, 2025). *ZO-1, Claudin-1*, and *Mucin-2* are associated with the intestinal mucosal barrier; *ZO-1* and *Claudin-1* are tight junction proteins and constitute structural components of the intestinal cellular barrier (S. Lu *et al.,* 2024). *Mucin-2* is one of the primary core components of mucins; it is secreted by goblet cells in the distal and proximal intestines and forms a barrier on the intestinal surface(Recktenwald *et al.,* 2024). In a study of colonic morphology in mice, the model group exhibited colonic tissue congestion and edema, indicating impaired intestinal barrier function. Compared to the model group, mice in the *Liparis nervosa* group exhibited reduced expression of the aforementioned substances, with expression levels falling between those of the control group and the model group. This suggests that *Liparis nervosa* can regulate the expression of inflammatory factors in the intestine to achieve an anti-inflammatory effect.

CONCLUSION

A 1% aqueous extract of *Liparis nervosa* was added to the fermentation medium, which was then clarified at 35°C and fermented for 4 days. Under these conditions, a high-quality aqueous extract of *Liparis nervosa* was obtained, possessing excellent functional and medicinal properties.

Liparis nervosa effectively alleviates the general symptoms of DSS-induced ulcerative colitis in mice, mitigates the adverse effects of the disease, and effectively suppresses the expression of inflammatory factors, indicating that *Liparis nervosa* has a therapeutic effect on ulcerative colitis.

Declaration of competing interest

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

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