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KYIV NATIONAL UNIVERSITY OF TECHNOLOGIES AND DESIGN
Faculty of Chemical and Biopharmaceutical Technologies
Department of Biotechnology, Leather and Fur

QUALIFICATION THESIS

on the topic **Study on active components and antioxidant activity of the *Platycodon grandiflorum* extract liquid fermented by *Lactobacillus rhamnosus***

First (Bachelor's) level of higher education
Specialty 162 "Biotechnology and Bioengineering"
Educational and professional program "Biotechnology"

Completed: student of group BEBT-20
Sun SHUQI

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Reviewer
Olena OKHMAT, Ph.D., As. prof.

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KYIV NATIONAL UNIVERSITY OF TECHNOLOGIES AND DESIGN

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Educational and professional program Biotechnology

APPROVE
Head of Department of Biotechnology, Leather and Fur, Professor,
Doctor of Technical Science
Olena MOKROUSOVA
_____
«___»_____2024

ASSIGNMENTS
FOR THE QUALIFICATION THESIS
Sun Shuqi

1. Thesis topic **Study on active components and antioxidant activity of the *Platycodon grandiflorum* extract liquid fermented by *Lactobacillus rhamnosus***
Scientific supervisor Olga Andreyeva, Dr. Sc., Prof.

approved by the order of KNUTD "__" _____2024, №____

2. Initial data for work: assignments for qualification thesis, scientific literature on the topic of qualification thesis, materials of Pre-graduation practice

3. Content of the thesis (list of questions to be developed): literature review; object, purpose, and methods of the study; experimental part; conclusions

4. Date of issuance of the assignments_____

EXECUTION SCHEDULE

№	The name of the stages of the qualification thesis	Terms of performance of stage	Note on performance
1	Introduction	From 01 April 2024 to 11 April 2024	
2	Section 1 Literature review	From 06 April 2024 to 20 April 2024	
3	Section 2 Object, purpose, and methods of the research	From 21 April 2024 to 30 April 2024	
4	Section 3 Experimental part	From 01 May 2024 to 10 May 2024	
5	Conclusions	From 07 May 2024 to 12 May 2024	
6	Draw up a bachelor's thesis (final version)	From 12 May 2024 to 24 May 2024	
7	Submission of qualification work to the supervisor for feedback (14 days before the defense)	From 24 May 2024 to 10 June 2024	
8	Submission of bachelor's thesis to the department for review (12 days before the defense)	13 June 2024	
9	Checking the bachelor's thesis for signs of plagiarism (10 days before the defense)	15 June 2024	
10	Submission of bachelor's thesis for approval by the head of the department (from 7 days before the defense)	17 June 2024	

I am familiar with the task:

Student _____ Sun SHUQI

Scientific supervisor. _____Olga ANDREYEVA

SUMMARY

Sun Shuqi. Study on active components and antioxidant activity of the Platycodon grandiflorum extract liquid fermented by Lactobacillus rhamnosus. – Manuscript.

Qualification thesis on the specialty 162 «Biotechnology and Bioengineering». – Kyiv National University of Technologies and Design, Kyiv, 2024.

Lactobacillus rhamnosus was inoculated into the extract liquid of Platycodon grandiflorum for fermentation, and the unfermented extract liquid was used as a control to analyze the effect of Lactobacillus rhamnosus fermentation on the active ingredients and antioxidant capacity of the Platycodon grandiflorum extract liquid.

The content of active ingredients such as flavonoids, polyphenols, and polysaccharides in the fermented extract of Platycodon grandiflorus increases. After 24 hours of fermentation, the flavonoid content rose by 115%, the polyphenol content rose by 121%, and the polysaccharide content rose by 164%. After 48 hours of fermentation, the flavonoid content rose by 120%, the polyphenol content rose by 131%, and the polysaccharide content rose by 165%. After 72 hours of fermentation, the flavonoid content rose by 123%, the polyphenol content rose by 130%, and the polysaccharide content rose by 160%. After 96 hours of fermentation, the flavonoid content rose by 125%, the polyphenol content rose by 120%, and the polysaccharide content rose by 161%; whereas the content of active ingredients in the control group remains relatively stable.

The scavenging capacities against DPPH free radical, hydroxyl radical, and superoxide anion radical in the fermented extract of Platycodon grandiflorus were significantly enhanced. Upon 24 hours of fermentation, the scavenging rate against DPPH free radical rose by 110%, that against hydroxyl radical by 110%, and that against superoxide anion radical by 162%; after 48 hours of fermentation, the scavenging rate against DPPH free radical rose by 116%, that against hydroxyl radical by 116%, and that against superoxide anion radical by 130%; after 72 hours of fermentation, the scavenging

rate against DPPH free radical rose by 120%, that against hydroxyl radical by 120%, and that against superoxide anion radical by 159%; and after 96 hours of fermentation, the scavenging rate against DPPH free radical rose by 119%, that against hydroxyl radical by 193%, and that against superoxide anion radical by 161%. In contrast, the antioxidant activity in the control group remained relatively stable.

From this, it can be seen that the active ingredients in the extract of Platycodon grandiflorus fermented by Lactobacillus rhamnosus are fully released, and the antioxidant capacity is significantly enhanced. Using this as a theoretical basis for developing new cosmetic raw materials, the study aims to enhance the utilization rate and added value of Platycodon grandiflorus.

Keywords: Platycodon grandiflorum, Lactobacillus rhamnosus, fermentation, active ingredients, antioxidant.

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INTRODUCTION

The relevance of the topic is the creation of a new probiotic-fermented material with high added value and biological activity.

Platycodonis Radix, commonly known as Air Flower root, comes from the plant Platycodon grandiflorum (Jacq) A.D.C., which belongs to the Campanulaceae family [1]. This root is rich in flavonoids, polyphenols, saponins and polysaccharides and is known for its powerful antioxidant, antitumor and anti-inflammatory properties. However, the current use of Platycodonis Radix is limited, resulting in a lack of diversity in processing methods and products, which in turn results in underutilization of the various components and their full range of benefits. Therefore, it is imperative to explore innovative processing methods to improve the functional use of Platycodonis Radix. Lactic acid bacteria (LAB) are known for their antitumor properties, ability to improve gut microbiota, and antioxidant functions [2]. Lactobacillus rhamnosus, a type of lactic acid bacteria, is an anaerobic and acid-resistant species that exhibits antioxidant properties and is non-pathogenic.

Taking into account the above, for more effective practical use of Platycodonis Radix, it is of interest to establish the feasibility of increasing its antioxidant activity as a result of fermentation with Lactic acid bacteria.

The purpose of the work is to study the active components and antioxidant activity of the liquid extract of Platycodon grandiflorum, fermented probiotic strain Lactobacillus rhamnosus.

The object of the study are the fermentation of the liquid extract of Platycodon grandiflorum with the probiotic strain Lactobacillus rhamnosus, as well as Platycodon grandiflorum and Lactobacillus rhamnosus themselves.

The subject of the study is to identify the active components and antioxidant activity of the liquid extract of Platycodon grandiflorum fermented by Lactobacillus rhamnosus.

The research method is visible spectrophotometry.

The scientific novelty of the work lies in the proof of the feasibility of fermentation of the liquid extract of Platycodon grandiflorum with the probiotic strain Lactobacillus rhamnosus, which consists in increasing the content of active ingredients (flavonoids, polyphenols and polysaccharides) and the rate of removal of DPPH radicals, hydroxyl radicals and superoxide anion radicals.

Practical significance of the results obtained. The human body contains a large number of free radicals that can use peroxidation to damage biological macromolecules such as proteins, cell membranes, enzymes, nucleic acids, etc. They can also cause lipid peroxidation reactions in unsaturated fatty acids, leading to a decrease in enzyme activity and mutations of DNA genes, which, in turn, damage cell functions, accelerate the aging of the body and harm the human body. Fermented Platycodon grandiflorus can be used as a basis for developing new probiotic fermented Platycodon grandiflorus raw materials with high added value and free radical scavenging activity.

CHAPTER 1
LITERATURE REVIEW

1.1 Research background

Platycodonis Radix, a perennial herb of the Campanulaceae family and a member of the Platycodon genus, is a plant that is both medicinal and edible. It is rich in active components such as flavonoids, polyphenols, and polysaccharides. Flavonoids, a type of polyphenolic compound widely distributed in nature, can form complexes with most metals, significantly enhancing their anti-inflammatory effects [3]. They also have inhibitory effects on enzyme activity, antibacterial and anti-allergic properties, and are non-toxic and harmless [4]. Polyphenols, important secondary metabolites widely found in plants, have strong antioxidant properties. They can eliminate various free radicals in the skin, slow down aging, and prevent skin cancer [5]. Polysaccharides, natural polymer compounds composed of more than 10 monosaccharide molecules linked by glycosidic bonds, have a wide range of biological activities, including antioxidant, anti-inflammatory, antibacterial, immune regulation, and inhibition of cancer cell proliferation [6].

Platycodon grandiflorus, rich in antioxidant active ingredients such as flavonoids and polyphenols, exhibits antioxidant properties. During the microbial fermentation process, enzymes produced by bacterial metabolism can affect the phenolic, flavonoid, and polysaccharide substances in Platycodon grandiflorus [7], converting large molecule glucoside compounds into small molecule aglycones, thereby improving the original antioxidant active ingredients in Platycodon grandiflorus and enhancing its antioxidant capacity [8].

Lactic acid bacteria (LAB) are gram-positive bacteria, cocci or rod-shaped bacteria. Peroxidase is negative, and the optimal growth condition is pH5.5-5.8. They are mainly found in dairy products, fruits, fermented meat and fish, fermented grains, Pickled vegetables, etc. In humans, lactic acid bacteria are mainly found in the mouth, colon, ileum, etc., and are widely distributed [9]. The internationally recognized

probiotic lactic acid bacteria that can coexist with the human body mainly include Streptococcus thermophilus, Lactobacillus rhamnosus, and Lactobacillus bulgaricus. Among them, Lactobacillus rhamnosus belongs to Lactobacillus, which is anaerobic, acid resistant, antioxidant, and nonpathogenic. It also has the function of regulating the balance of microorganisms and microbiota in the host's intestinal tract, thereby enhancing the host's intestinal resistance, reducing or eliminating toxins, and treating intestinal diseases [10].

Studies have shown that the fermentation process of lactic acid bacteria typically produces metabolites with biological activity, preventing infection, immune regulation, antioxidant, and anti-anxiety effects. Lactic acid bacteria fermented foods also have effects such as enhancing mineral absorption, alleviating lactose intolerance, and inhibiting antibiotic-induced diarrhea. Moreover, lactic acid bacteria fermentation can facilitate the release of effective components in traditional Chinese herbs [11]. During the fermentation process, Lactobacillus rhamnosus can produce active ingredients that inhibit bacterial growth, preventing food spoilage and the proliferation of pathogens. A trace amount of Lactobacillus rhamnosus fermentation broth extract can significantly inhibit common pathogenic bacteria in the skin, such as Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa [12].

1.2 Current research status at home and abroad

1.2.1 Current research status abroad

In South Korea, the extract of Platycodon grandiflorus, commonly known as balloon flower, is extensively utilized in the cosmetics industry. The anti-inflammatory and antioxidant properties of its active components, such as platycodon saponins and flavonoids, are highly effective in reducing skin damage, decelerating the aging process, and maintaining the smoothness of the skin [13]. Zou and colleagues [14] have isolated a pectin polysaccharide, designated as PGP-I-I, from the roots of this traditional medicinal plant. In vitro studies revealed that under conditions of hydrogen peroxide treatment, PGP-I-I is capable of restoring the antioxidant defense system of

intestinal cells by enhancing the expression of cellular antioxidant genes, thereby exerting a protective effect against oxidative damage. Additionally, the petroleum ether extract derived from Platycodon grandiflorus has been confirmed to possess both antioxidant and anti-cancer activities, with the antioxidant activity being closely associated with the levels of phenolic compounds present [15]. Concurrently, research has demonstrated that the extract of Platycodon grandiflorus can be effectively employed in the treatment of ulcerative colitis and atopic dermatitis in mice induced by sodium dextran sulfate, and it has shown promising therapeutic effects on mice with melasma [16].

1.2.2 Current research status in China

Research by Ai Su and colleagues [17] indicates that fermenting Platycodon grandiflorus with microorganisms can significantly enhance the release of its active substances. The concentration of flavonoids, saponins, and polysaccharides in the post-fermentation extract is markedly increased. Liu Hua-li and team [18] optimized the extraction process of Platycodon polyphenols using an orthogonal design method and measured their antioxidant activity. Platycodon polyphenols have been found to possess scavenging activity against superoxide anions and hydroxyl radicals, with their scavenging capacity strengthening as the concentration of polyphenols increases. Wang Xiao-lin and others [19] refined the process for purifying total flavonoids from Platycodon stems using microporous adsorption resin and simultaneously assessed their in vitro antioxidant activity. The extract of total flavonoids from Platycodon stems demonstrated strong scavenging capabilities against DPPH· and ·OH radicals, with the scavenging activity progressively enhancing as the mass concentration of the extracted total flavonoids from Platycodon stems increased.

Conclusions to chapter 1

1. Platycodon grandiflorum and Lactobacillus rhamnosus both have antioxidant effects, and their functions can be used comprehensively to enhance antioxidant capabilities.
2. The active components in Platycodon grandiflorum, such as flavonoids, polyphenols, polysaccharides, and others, all have antioxidant effects.

CHAPTER 2
OBJECT, PURPOSE, AND METHODS OF THE STUDY

2.1 Object of the study

The object of the study are the fermentation of the liquid extract of Platycodon grandiflorum with the probiotic strain Lactobacillus rhamnosus, as well as Platycodon grandiflorum and Lactobacillus rhamnosus themselves.

2.2 Purpose of the study

The study employs the probiotic strain Lactobacillus rhamnosus for the fermentation of Platycodon grandiflorum extract. The purpose of the work is to study the active components and antioxidant activity of the liquid extract of Platycodon grandiflorum, fermented probiotic strain Lactobacillus rhamnosus.

2.3 Methods of the study

Research method is visible spectrophotometry.

Spectroscopy is a spectral analysis method that utilizes the absorption characteristics of substances to qualitatively and quantitatively analyze light. UV spectrophotometry is a type of spectrophotometry. UV spectrophotometry is a method of qualitative and quantitative analysis that utilizes the absorption characteristics of substances in the ultraviolet region (200-400 nm).

UV spectrophotometry is based on Lambert Beer's law, which states that the absorbance of a solution (A) is directly proportional to the product of the concentration of the solution (C) and the thickness of the liquid layer (b), $A = \varepsilon * C * b$. In the formula, ε is the molar absorptivity, indicating the sensitivity of the substance that absorbs light. Ultraviolet-visible (UV-Vis) spectrophotometry is a quick, sensitive, and user-friendly analytical technique, applicable for the detection and analysis of a wide range of substances.

2.3.1 Experimental materials and equipment

2.3.1.1 Experimental materials

Platycodon grandiflorum, commonly known as the balloon flower root, is derived from the plant Platycodon grandiflorum (Jacq) A. D. C., which belongs to the Campanulaceae family. This root is rich in flavonoids, polyphenols, saponins, and polysaccharides, and is known for its potent antioxidant, anti-tumor, and anti-inflammatory properties.

2.3.1.2 Bacterial strain

Lactobacillus rhamnosus 217-1

Lactic acid bacteria (LAB) are known for their anti-tumor properties, their ability to improve gut microbiota, and their antioxidant functions. Lactobacillus rhamnosus, a type of LAB, is an anaerobic and acid-tolerant species that exhibits antioxidant properties and is non-pathogenic.

2.3.1.3 Main reagents

Yeast powder, glucose, peptone, triammonium citrate, manganese sulfate, anhydrous sodium acetate, disodium hydrogen phosphate, potassium acetate, beef paste, Tween-80, rutin, ethanol, $NaNO_2$, Al (NO) $_3$, NaOH, gallic acid, folinol reagent, Na_2CO_3, glucose, phenol, concentrated sulfuric acid; Ascorbic acid (Vc), DPPH, ethanol, $FeSO_4$, ortho phenanthrene, H_2O_2, Tris HCL, pyrogallol, anhydrous sodium phosphate, ammonium molybdate tetrahydrate.

2.3.1.4 Experimental instruments

Centrifuge; Electronic analytical balance; UV visible spectrophotometer; Constant temperature water bath pot; Constant temperature incubator; PH meter.

2.4 Experimental methods

2.4.1 Preparation of the Fermentation Broth

Lactobacillus rhamnosus is inoculated into MRS liquid medium at a ratio of 2% (v/v) and incubated statically in an incubator at 37°C overnight. The dried Platycodon grandiflorum root slices are ultra-micro pulverized into a powder form. The fermentation substrate is prepared by adding 0.5 g of yeast powder and 2 g of glucose to every 100 mL of ultrapure water. To this substrate, 5 g of the pulverized Platycodon powder is added and mixed thoroughly, followed by low-temperature sterilization at 85 °C for 30 minutes. Once the fermentation medium has cooled to room temperature, Lactobacillus rhamnosus is inoculated at a ratio of 2% (v/v) and the culture is maintained at a constant temperature of 37 °C. A control group is also prepared by sterilizing the substrate and not inoculating it with the bacterial strain. In triplicate for each group, samples are taken every 24 hours, centrifuged at 10,000 rpm for 5 minutes, and the supernatant is collected and stored at 4 °C for further use.

2.4.2 Sample Preparation

Under aseptic conditions, samples are collected every 24 hours to monitor the changes in pH and the content of active components in both the fermentation and control groups over a period of 96 hours.

2.4.3 Determination of pH Values in Fermentation and Control Groups

The pH values of the fermentation broth and the control group are measured using a pH meter.

Basic Usage of a pH Meter:

1. Calibrate the pH Meter: Before using it, you need to calibrate the pH meter to ensure the accuracy of the measurements. Use standard buffer solutions (typically pH 4.00, 7.00, and 10.00) for calibration. Follow the instruction manual of the pH meter for multi-point calibration, usually requiring at least two standard solutions with different pH values.

2. Clean the pH Electrode: Make sure the pH electrode is clean and free of residues before measuring. Wash the electrode with distilled water, then gently wipe it with a soft non-woven cloth or tissue.

3. Immerse in the Sample: Submerge the pH electrode into the solution to be tested, ensuring it is fully immersed. Avoid the electrode touching the walls or bottom of the container.

4. Wait for a Stable Reading: Wait for the value shown on the pH meter to stabilize. Some pH meters have an automatic stabilization feature that alerts when the value is stable.

5. Record the pH Value: Once the reading is stable, record the pH value displayed on the pH meter.

6.Precautions:

Avoid using the pH meter in extreme temperatures.

Do not allow the pH electrode to dry out, as this will reduce its service life.

After measuring solutions of strong acids or strong bases, rinse the electrode thoroughly with distilled water.

2.4.4 Construction of the Flavonoid Standard Curve

The total flavonoid content in the samples is determined using the aluminum nitrate spectrophotometric method [20]. Accurately weigh 5 mg of the reference compound rutin and dissolve it in 70% ethanol to prepare a 0.2 mg/mL rutin solution in a 25 mL volumetric flask. Transfer 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, and 0.7 mL of the rutin solution into separate test tubes. Add 70% ethanol to each tube to make up a volume of 2 mL. Then, add 0.3 mL of 5% $NaNO_2$ solution, shake well, and let stand for 6 minutes. Subsequently, add 0.3 mL of 10% Al $(NO_3)_3$ solution, shake well, and let stand for another 6 minutes. Finally, add 2 mL of 4% NaOH solution, shake well, and let stand for 10 minutes. Measure the absorbance (A) at a wavelength of 510 nm and plot the standard curve.

Usage Method of UV-Vis Spectrophotometer:

1. Power On and Preheat: Turn on the instrument and allow it to warm up for a while, typically around 20 minutes, to ensure stability.

2. Select the Light Source: Choose the appropriate light source according to the experimental requirements, such as a hydrogen lamp, deuterium lamp, or tungsten lamp.

3. Wavelength Setting: Adjust the wavelength dial or enter the desired wavelength through the software to ensure the accuracy of the monochromatic light.

4. Calibrate the Instrument: Use the automatic calibration feature or manually calibrate the instrument according to the manual to ensure measurement accuracy.

5. Zero Adjustment: Adjust to zero using a cuvette filled with distilled water, ensuring the instrument is at the baseline before measurement.

6. Measure the Sample: Place the sample into the sample compartment and perform the test, noting the absorbance values.

7. Data Processing: Use the device's built-in functions or external software to plot a standard curve for quantitative analysis.

8. Cleaning and Maintenance: After the measurement is complete, clean the cuvettes and maintain the instrument as necessary.

Precautions: Make sure the cuvettes are clean, avoid using expired standard solutions, and regularly calibrate the instrument to ensure the accuracy of the measurements.

2.4.5 Determination of Flavonoid Content in the Fermentation and Control Groups

Take 1 mL of the sample solution and add 1 mL of 70% ethanol. If the absorbance is too high, dilute the sample appropriately and then proceed with the measurement as described above. Each sample should be measured three times and the average value should be taken. Calculate the total flavonoid content in the samples from the standard curve.

2.4.6 Construction of the Polyphenol Standard Curve

The content of polyphenols is determined using the Folin-Ciocalteu method [21]. Accurately weigh 5 mg of the gallic acid reference standard and dissolve it in distilled water to prepare a 0.1 mg/mL gallic acid solution in a 50 mL volumetric flask. Transfer 0, 1, 2, 3, 4, 5, 6, and 7 mL of the gallic acid solution into separate test tubes and adjust the volume to 10 mL with distilled water to create a series of gallic acid solutions with concentrations of 0, 10, 20, 30, 40, 50, 60, and 70 μg/mL. To each test tube, add 5 mL of distilled water, 1 mL of Folin-Ciocalteu reagent, and 3 mL of sodium carbonate solution (0.075 g/mL). After shaking to mix, let the mixture stand for 1 hour to develop the color. Measure the absorbance (A) at a wavelength of 760 nm and plot the standard curve.

2.4.7 Determination of Polyphenol Content in the Fermentation and Control Groups

Dilute the sample as necessary and take 1 mL of the diluted sample. Follow the steps outlined above to add the reagents and measure the absorbance (A). Perform the experiment in triplicate and take the average value. Calculate the total polyphenol content in the samples using the standard curve.

2.4.8 Construction of the Polysaccharide Standard Curve

The content of polysaccharides is determined using the phenol-sulfuric acid method. Accurately weigh 5 mg of the glucose reference standard and dissolve it in distilled water to prepare a 1 mg/mL glucose solution in a 50 mL volumetric flask. Transfer 0, 0.2, 0.4, 0.6, 0.8, and 1.0 mL of the glucose solution into separate test tubes and adjust the volume to 1 mL with distilled water. To each test tube, add 1 mL of 5% (v/v) phenol solution and then quickly add 5 mL of concentrated sulfuric acid. Allow the mixture to stand for 10 minutes, then place the test tubes in a 30°C water bath for 20 minutes. After cooling, measure the absorbance (A) at a wavelength of 490 nm and plot the standard curve.

2.4.9 Determination of Polysaccharide Content in the Fermentation and Control Groups

Dilute the sample as necessary and take 1 mL of the diluted sample. Follow the steps outlined above to add the reagents and measure the absorbance (A). Perform the experiment in triplicate and take the average value. Calculate the total polysaccharide content in the samples using the standard curve.

2.4.10 DPPH Radical Scavenging Activity Assay

Place 1 mL of the sample solution into a light-protected centrifuge tube. Add 3 mL of DPPH solution (0.5 mM prepared in anhydrous ethanol on ice), mix well, and allow the mixture to stand in the dark for 30 minutes. After centrifugation at 10,000 rpm for 5 minutes, measure the absorbance at 517 nm using a spectrophotometer. For the blank group, add 1 mL of culture medium and 3 mL of anhydrous ethanol. The control group consists of 1 mL of sterile water and 3 mL of DPPH solution prepared in anhydrous ethanol. Ascorbic acid (Vc) is used as a positive control. The DPPH scavenging rate (%) is calculated using the formula:

$$\text{Scavenging Rate} = [\ 1- (A_S - A/A_0\ )\ ] \times 100 \quad (2.1)$$

where A_S is the absorbance of the sample group, A_0 is the absorbance of the control group, and A is the absorbance of the blank group.

2.4.11 Hydroxyl Radical Scavenging Activity Assay

To each test tube, add 0.5 mL of the sample solution, 1 mL of 0.02 mmol/L PBS (pH=7.4), 0.5 mL of 2.5 mmol/L $FeSO_4$, and 0.5 mL of 2.5 mmol/L o-phenylenediamine. Initiate the reaction by adding 0.5 mL of 20 mmol/L H_2O_2, then incubate the mixture in a 37°C water bath for 1 hour. After the reaction, centrifuge the mixture at 4,000 rpm for 10 minutes. Use distilled water as a blank control. Measure the absorbance of the fermentation liquid at a wavelength of 536 nm. The calculation formula for the scavenging rate is:

$$\text{Scavenging Rate} = [(A_{sample} - A_{control}) / (A_{blank} - A_{control})] \times 100 \quad (2.2)$$

where A_{sample} is the absorbance after the addition of the sample solution, A_{blank} is the background absorbance measured when using distilled water instead of the sample and H_2O_2 at the corresponding concentration, and $A_{control}$ is the absorbance when using distilled water instead of the sample.

2.4.12 Superoxide Anion Radical Scavenging Activity Assay

Prepare the reaction mixtures as follows:

For A_s (Sample with Tris-HCl and Catechol): Mix the sample solution, Tris-HCl solution, and catechol solution in a ratio of 0.6:2:0.4.

For A_b (Sample with Tris-HCl and PBS): Mix the sample solution, Tris-HCl solution, and PBS solution in a ratio of 0.6:2:0.4.

For A_0 (Catechol with Tris-HCl and PBS): Mix catechol solution, Tris-HCl solution, and PBS solution in a ratio of 0.4:2:0.6.

Add the prepared reaction mixtures to light-protected centrifuge tubes and incubate at 25°C for 20 minutes. Use ascorbic acid solution as a positive control and incubate at 25°C for 20 minutes. Measure the absorbance at 325 nm.

The superoxide anion radical scavenging rate (%) is calculated using the formula:

$$\text{Scavenging Ratec} = [\ 1- (A_S - A_b/A_0\ )\ ] \times 100 \qquad (2.3)$$

2.4.13 Total Antioxidant Capacity Assay

Mix 3 mL of the ammonium molybdate reagent system (28 mM disodium hydrogen phosphate, 4 mM ammonium molybdate tetrahydrate, and concentrated sulfuric acid, prepared separately and then mixed in equal parts) with 0.3 mL of the sample solution. Incubate the mixture in a 95°C water bath for 90 minutes. After cooling to room temperature, centrifuge at 10,000 rpm for 5 minutes. Measure the absorbance at 695 nm. Use ascorbic acid (Vc) as a positive control to compare the total antioxidant capacity based on the absorbance values.

Conclusions to chapter 2

1. When determining the content of active components in Platycodon grandiflorum, it is necessary to first plot a standard curve through experiments and then measure the specific content.
2. The antioxidant capacity can be determined by measuring the rate of free radical scavenging, and there are various methods to assess antioxidant capacity.

CHAPTER 3
EXPERIMENTAL PART

3.1 Detection of pH Values in Fermentation and Control Groups

The changes in pH values of the fermentation and control groups are depicted in Figure 3.1. The pH value of the Platycodon grandiflorus extract liquid fermented with Lactobacillus rhamnosus is significantly lower than that of the control group, indicating a higher acidity in the fermentation group. When the Platycodon grandiflorus extract liquid inoculated with Lactobacillus rhamnosus is cultured under static conditions, the bacteria primarily undergo anaerobic respiration, leading to acid production. Furthermore, the content of active components such as flavonoids and polyphenols in the fermented Platycodon grandiflorus extract increases. These active components exhibit acidity, which contributes to the lower pH value of the fermentation liquid compared to the control group.

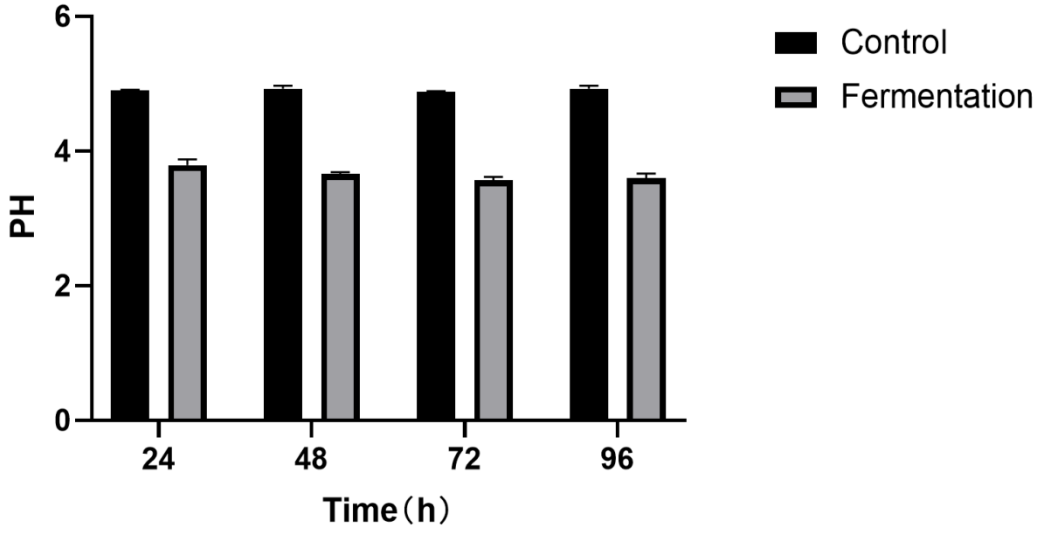

Figure 3.1 – **Fermentation and Control Group pH Value Variation**

3.2 Flavonoid Standard Curve Construction

The standard curve of flavonoid is shown in Figure 3.2.

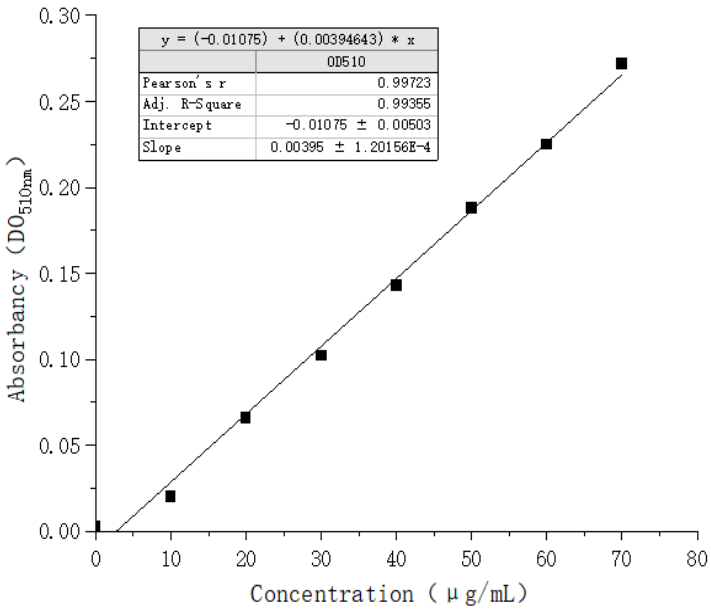

Figure 3.2 – **Flavonoid Standard Curve**

3.3 Detection of Flavonoid Content Variation in Fermentation and Control Groups

The variations in flavonoid content within the fermentation and control groups are depicted in Figure 3.3. Upon inoculation with Lactobacillus rhamnosus, the content of flavonoid in the fermented Platycodon grandiflorus extract progressively increases over the course of the fermentation period. Notably, the highest increase in flavonoid content is observed within the first 96 hours of fermentation.

Specifically, after 24 hours of fermentation, the flavonoid content increased by 115%; at 48 hours, the increase reached 120%; at 72 hours, the content rose by 123%; and at 96 hours, the growth in content reached 125%. In contrast, the control group without inoculation of Lactobacillus rhamnosus showed relatively stable flavonoid content. This outcome suggests that Lactobacillus rhamnosus has a positive effect on promoting the release of active components in Platycodon grandiflorum.

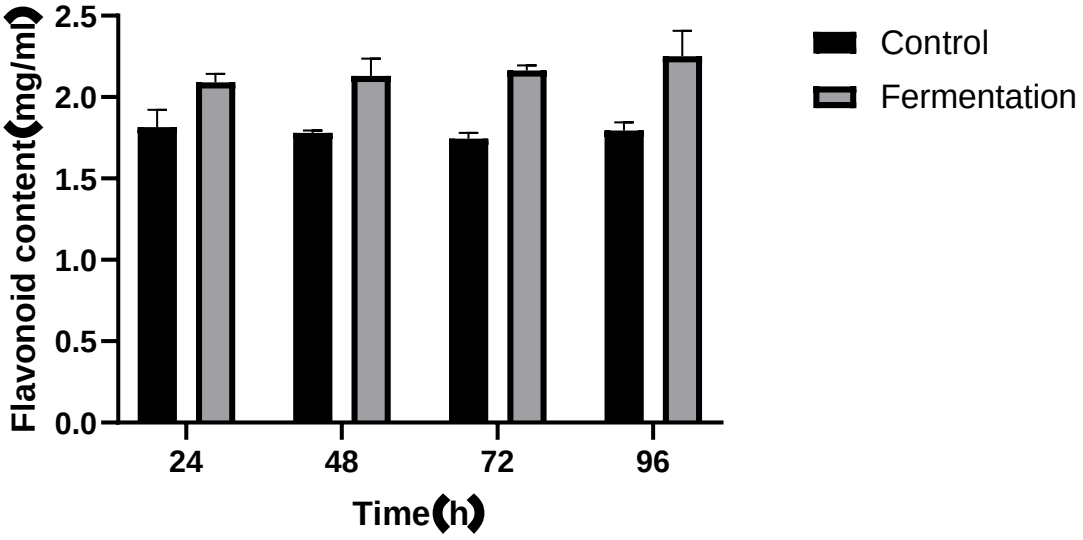

Figure 3.3 – **Variation in Flavonoid Content in Fermentation and Control Groups**

3.4 Polyphenol Standard Curve Construction

The standard curve of polyphenol is shown in Figure 3.4.

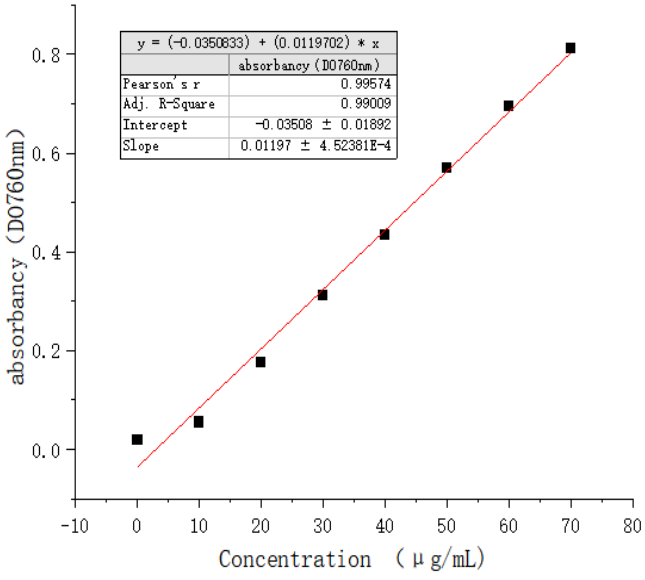

Figure 3.4 – **Polyphenol Standard Curve**

3.5 Detection of Polyphenol Content Variation in Fermentation and Control Groups

The variations in polyphenol content within the fermentation and control groups are depicted in Figure 3.5. Upon inoculation with Lactobacillus rhamnosus, the content of polyphenol in the fermented Platycodon grandiflorus extract progressively increases over the course of the fermentation period. Notably, the highest increase in polyphenol content is observed within the first 96 hours of fermentation.

After 24 hours of fermentation, the relative increase rate of polyphenol content reached 121%; after 48 hours, this ratio further rose to 131%; at 72 hours, the relative increase rate of polyphenol content was 130%; and at 96 hours, the relative increase rate slightly decreased to 120%. In contrast, in the control group without inoculation of Lactobacillus rhamnosus, the polyphenol content remained at a relatively stable level. This phenomenon indicates that Lactobacillus rhamnosus plays a significant role in promoting the biotransformation and release of active polyphenolic substances in Platycodon grandiflorum extract.

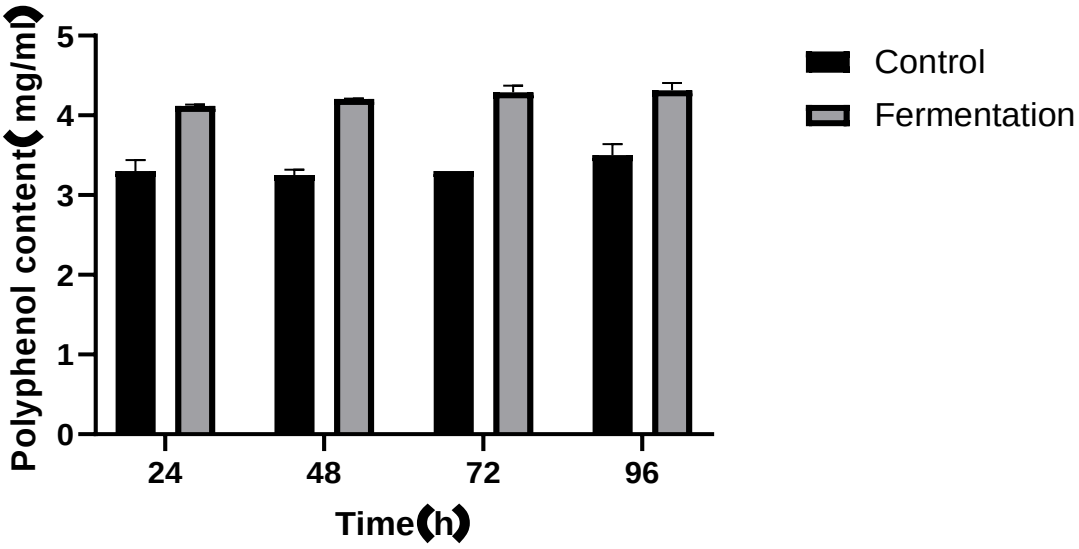

Figure 3.5 – **Variation in Polyphenol Content in Fermentation and Control Groups**

3.6 Polysaccharide Standard Curve Construction

The standard curve of Polysaccharide is shown in Figure 3.6.

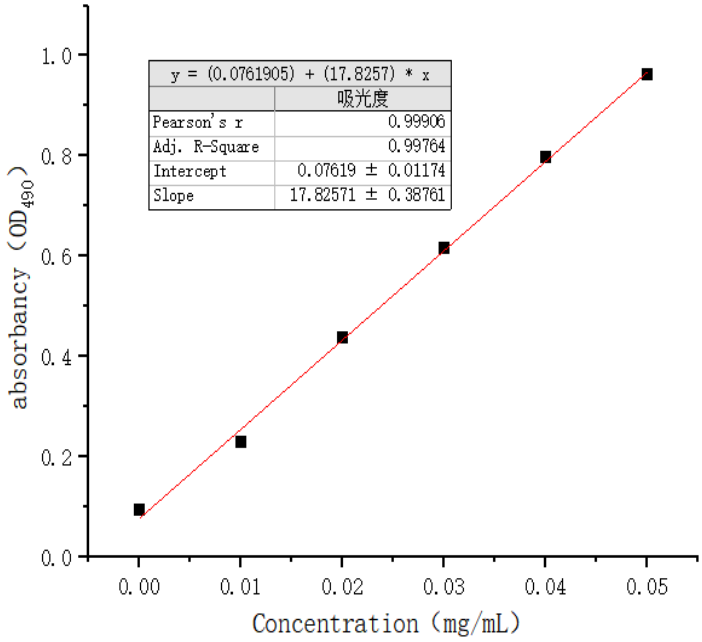

Figure 3.6 – **Polysaccharide Standard Curve**

3.7 Detection of Polysaccharide Content Variation in Fermentation and Control Groups

The variations in Polysaccharide content within the fermentation and control groups are depicted in Figure 3.7. Upon inoculation with Lactobacillus rhamnosus, the content of Polysaccharide in the fermented Platycodon grandiflorus extract progressively increases over the course of the fermentation period. Notably, the highest increase in Polysaccharide content is observed within the first 96 hours of fermentation.

Specifically, after 24 hours of fermentation, the relative increase in polysaccharide content was 164%; after 48 hours, the increase marginally rose to 165%; at 72 hours, the relative increase slightly declined to 160%; and at 96 hours, the relative increase was 161%. In contrast, the polysaccharide content in the control group without the inoculation of Lactobacillus rhamnosus remained relatively constant. This comparative result suggests that Lactobacillus rhamnosus has played a pivotal role in

facilitating the biosynthesis and accumulation of active polysaccharide components in the extract of Platycodon grandiflorum.

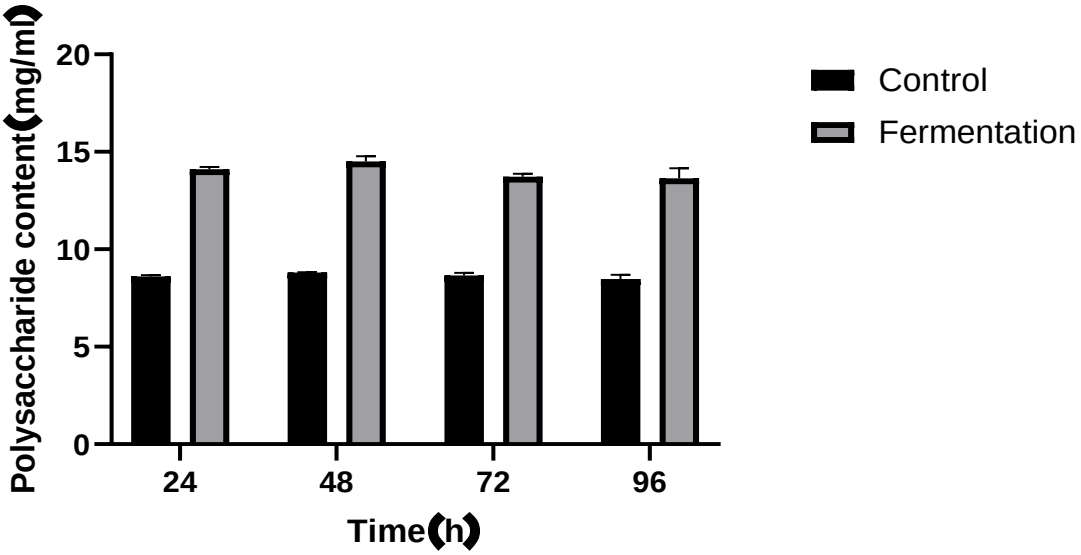

Figure 3.7 – **Variation in Polysaccharide Content in Fermentation and Control Groups**

3.8 DPPH Radical Scavenging Capacity

The influence of Lactobacillus rhamnosus fermentation on the DPPH (2,2-Diphenyl-1-picrylhydrazyl) free radical scavenging capacity of the Platycodon grandiflorus extract is illustrated in Figure 3.8. The results demonstrate a noticeable enhancement in the DPPH free radical scavenging ability of the extract following fermentation with Lactobacillus rhamnosus.

Specifically, after 24 hours of fermentation, the DPPH free radical scavenging rate of the Platycodon grandiflorum extract significantly increased by 110%; after 48 hours, the rate further enhanced to 116%; at 72 hours, the scavenging rate increased to 120%; and at the end of the 96-hour fermentation period, the DPPH free radical scavenging rate was sustained at a high level of 119%. These data consistently demonstrate that the fermentative action of Lactobacillus rhamnosus significantly promotes the antioxidant potential of the Platycodon grandiflorum extract.

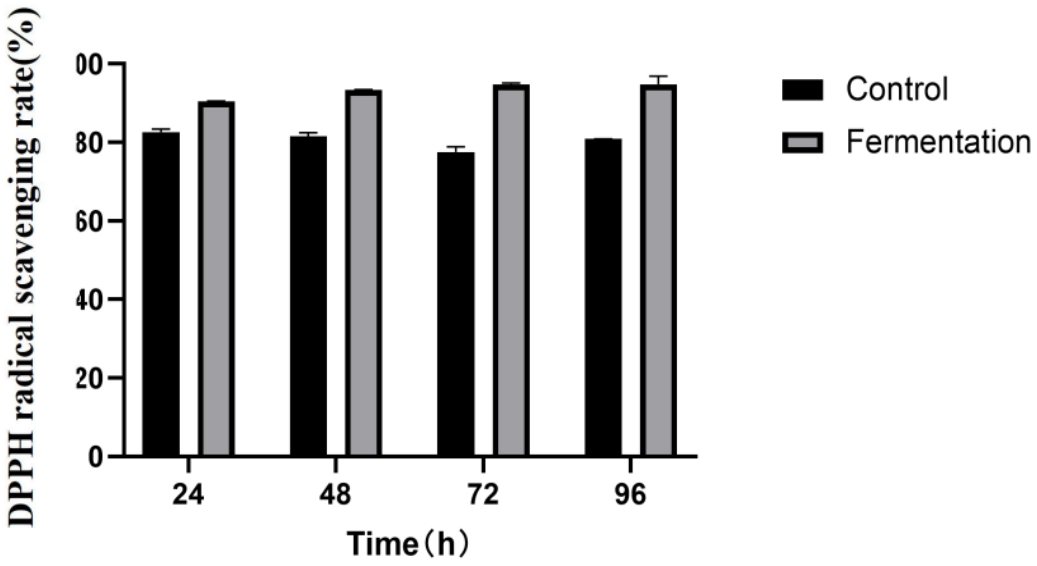

Figure 3.8 – **Influence of Lactobacillus rhamnosus fermentation on the DPPH free radical scavenging capacity of the Platycodon grandiflorus extract**

3.9 Hydroxyl Radical Scavenging Capacity

The influence of Lactobacillus rhamnosus fermentation on the Hydroxyl free radical scavenging capacity of the Platycodon grandiflorus extract is illustrated in Figure 3.9. The results demonstrate a noticeable enhancement in the Hydroxyl free radical scavenging ability of the extract following fermentation with Lactobacillus rhamnosus.

Specific data indicates that after 24 hours of fermentation, compared to the control group, the hydroxyl radical scavenging rate of the Platycodon grandiflorum extract in the experimental group significantly increased by 110%; after 48 hours, this rate further strengthened to 116%; at 72 hours, the scavenging rate rose to 120%. At the end of the 96-hour fermentation period, the hydroxyl radical scavenging rate in the experimental group significantly increased to 193%. This result suggests that as the fermentation time extends, the antioxidant activity of the Platycodon grandiflorum extract is further enhanced. In summary, the fermentation effect of Lactobacillus rhamnosus has a

significant promoting effect on enhancing the hydroxyl radical scavenging capacity of the Platycodon grandiflorum extract.

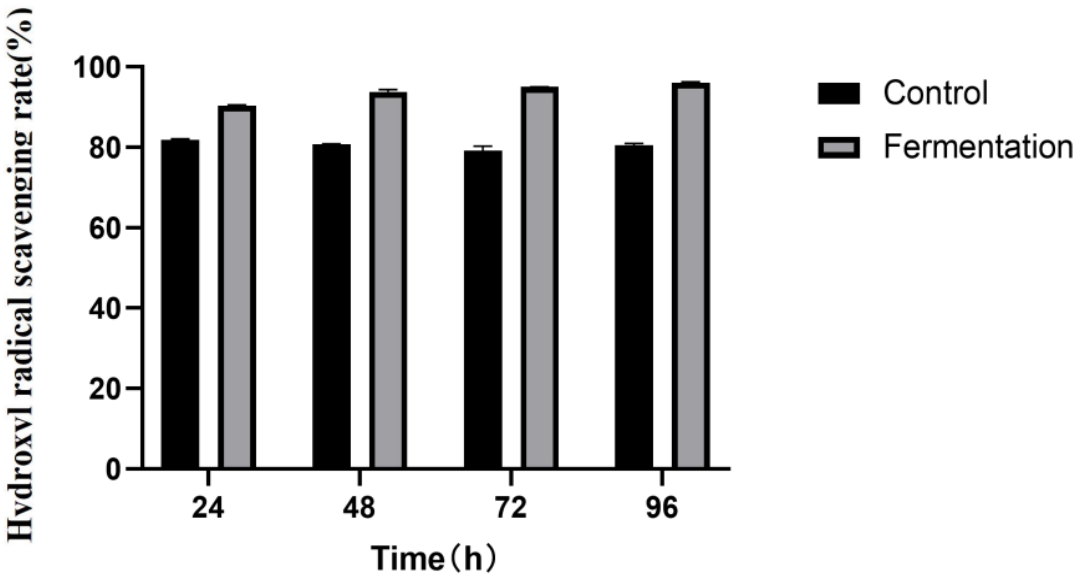

Figure 3.9 – **Influence of Lactobacillus rhamnosus fermentation on the Hydroxyl free radical scavenging capacity of the Platycodon grandiflorus extract**

3.10 Superoxide Anion Free Radical Scavenging Capacity

The influence of Lactobacillus rhamnosus fermentation on the Superoxide anion free radical scavenging capacity of the Platycodon grandiflorus extract is illustrated in Figure 3.10. The results demonstrate a noticeable enhancement in the Superoxide anion free radical scavenging ability of the extract following fermentation with Lactobacillus rhamnosus.

Specific data shows that after 24 hours of fermentation, compared to the control group, the superoxide anion radical scavenging rate in the experimental group significantly increased by 162%; after 48 hours, the scavenging rate declined somewhat but still remained at a level 130% higher than the control group; after 72 hours, the scavenging rate rose again to 159% more than the control group; and by the 96-hour mark, the scavenging rate further increased to 161% above the control group. In summary, the fermentative action of Lactobacillus rhamnosus has a significant

promoting effect on enhancing the hydroxyl radical scavenging capacity of the Platycodon grandiflorum extract.

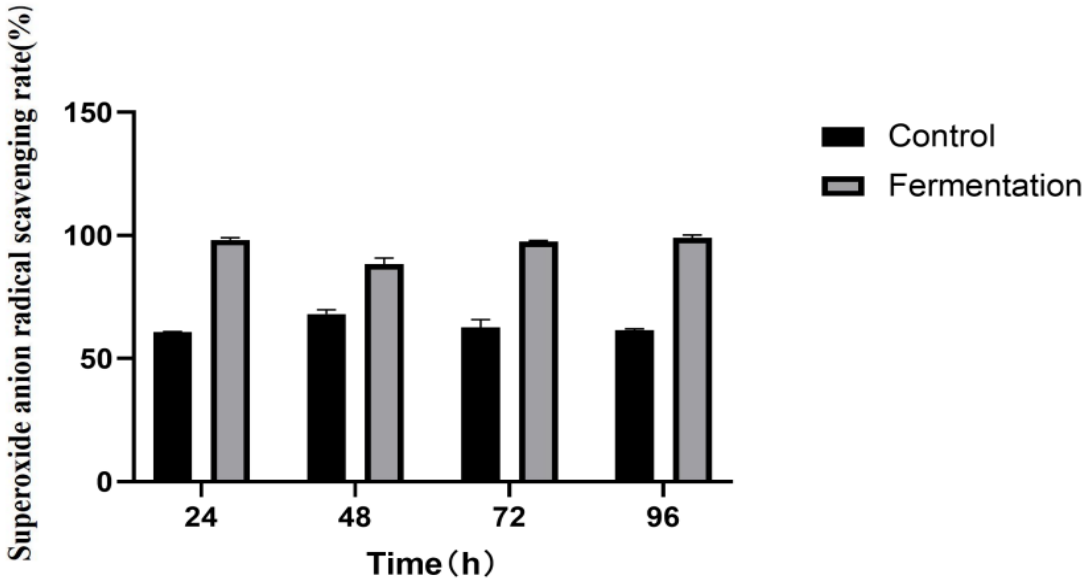

Figure 3.10 – **Influence of Lactobacillus rhamnosus fermentation on the Superoxide anion free radical scavenging capacity of the Platycodon grandiflorus extract**

3.11 Total Antioxidant Capacity

The influence of Lactobacillus rhamnosus fermentation on the total antioxidant capacity of the Platycodon grandiflorus extract is illustrated in Figure 3.11. The extract of Platycodon grandiflorus fermented by Lactobacillus rhamnosus and its total antioxidant capacity decreased.

In contrast to the control group, the total antioxidant capacity of the experimental group was reduced by 68% after 24 hours of fermentation, by 74% after 48 hours of fermentation, sustained a 74% decrease after 72 hours of fermentation, and was lowered by 73% after 96 hours of fermentation.

The results of the DPPH free radical scavenging, superoxide anion test, and hydroxyl radical experiment were all positive, indicating that the fermentation of Platycodon grandiflorum by Lactobacillus rhamnosus can promote the antioxidant capacity of its fermentation supernatant. However, the total antioxidant experiment

yielded negative results, which may be attributed to the complex interactions of multiple factors:

1. Diversity of Free Radical Types: The DPPH test, hydroxyl radical test, and superoxide anion test target specific free radicals, whereas the total antioxidant experiment may cover a broader range of free radical types and reactive oxygen species (ROS). This can lead to samples that perform well in specific free radical tests but may not show ideal scavenging effects when faced with multiple free radicals in the total antioxidant capacity test.

2. Specificity of Antioxidant Components: Some components, such as certain flavonoids, may exhibit strong scavenging ability in the DPPH free radical test but may not be as effective in scavenging hydrogen peroxide or other ROS.

3. Experimental Conditions: Differences in experimental conditions, such as pH value, temperature, and reaction time, can also affect the activity and stability of antioxidant components, thereby influencing the experimental results.

4. Synergistic Effects of Antioxidant Components: The synergistic effects between antioxidant components are crucial in the total antioxidant experiment. If components that efficiently scavenge DPPH free radicals cannot effectively work in synergy with other components, the overall antioxidant effect may be negatively impacted.

It's important to consider these factors when interpreting the results of antioxidant experiments to gain a comprehensive understanding of the antioxidant properties of a substance.

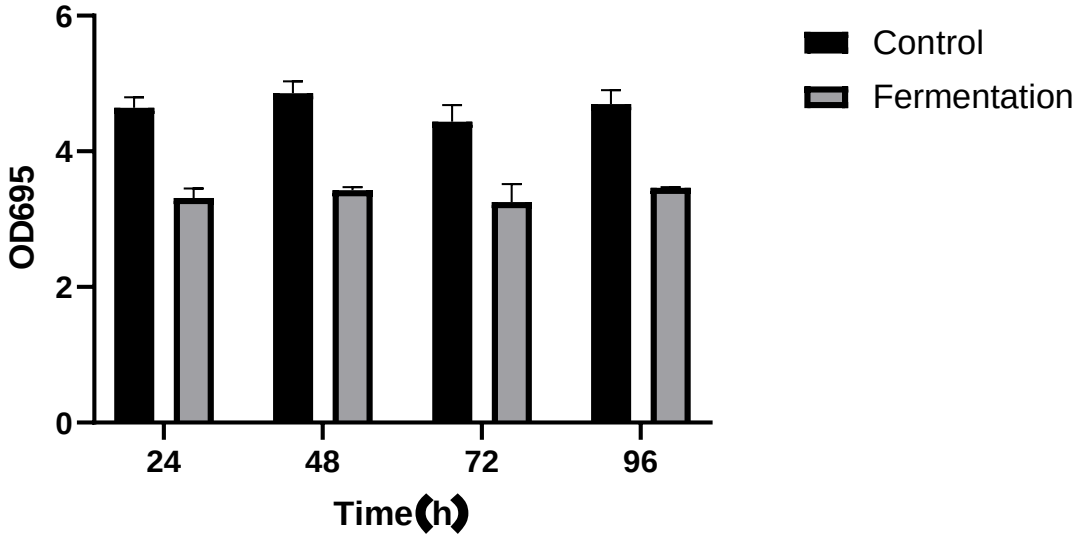

Figure 3.11 – Influence of Lactobacillus rhamnosus fermentation on the total antioxidant capacity of the Platycodon grandiflorus extract

Conclusions to chapter 3

1. The pH value of the Platycodon grandiflorus extract liquid fermented with Lactobacillus rhamnosus is significantly lower than that of the control group, indicating a higher acidity in the fermentation group.
2. Upon inoculation with Lactobacillus rhamnosus, the content of flavonoid in the fermented Platycodon grandiflorus extract progressively increases over the course of the fermentation period. Notably, the highest increase in flavonoid content is observed within the first 96 hours of fermentation. Specifically, the flavonoid content exhibited a substantial increase, reaching 115% higher at the 24-hour mark, escalating further to 120% at 48 hours, and continuing this upward trend to 123% at 72 hours, and ultimately achieving a 125% increase at the 96-hour interval. These figures stand in stark contrast to the control group, which did not undergo fermentation with Lactobacillus rhamnosus and maintained a relatively stable flavonoid content throughout the observation period.

3. Upon inoculation with Lactobacillus rhamnosus, the content of Polysaccharide in the fermented Platycodon grandiflorus extract progressively increases over the course of the fermentation period. Notably, the highest increase in Polysaccharide content is observed within the first 96 hours of fermentation. Specifically, the flavonoid content exhibited a substantial increase, reaching 121% higher at the 24-hour mark, escalating further to 131% at 48 hours, and continuing this upward trend to 130% at 72 hours, and ultimately achieving a 120% increase at the 96-hour interval. These figures stand in stark contrast to the control group, which did not undergo fermentation with Lactobacillus rhamnosus and maintained a relatively stable flavonoid content throughout the observation period.
4. Upon inoculation with Lactobacillus rhamnosus, the content of Polysaccharide in the fermented Platycodon grandiflorus extract progressively increases over the course of the fermentation period. Notably, the highest increase in Polysaccharide content is observed within the first 96 hours of fermentation. Specifically, the flavonoid content exhibited a substantial increase, reaching 164% higher at the 24-hour mark, escalating further to 165% at 48 hours, and continuing this upward trend to 160% at 72 hours, and ultimately achieving a 161% increase at the 96-hour interval. These figures stand in stark contrast to the control group, which did not undergo fermentation with Lactobacillus rhamnosus and maintained a relatively stable flavonoid content throughout the observation period.
5. The DPPH free radical scavenging ability of Platycodon grandiflorus extract fermented by Lactobacillus rhamnosus is improved. After 24 hours of fermentation, the DPPH free radical scavenging rate increased by 110%. After 48 hours of fermentation, the DPPH free radical scavenging rate increased by 116%. After 72 hours of fermentation, the DPPH free radical scavenging rate increased by 120%. After 96 hours of fermentation, the DPPH free radical scavenging rate increased by 119%. This indicates that the

fermentation of Lactobacillus rhamnosus can improve the DPPH free radical scavenging ability of Platycodon grandiflorus.

6. The hydroxyl radical scavenging ability of the extract of Platycodon grandiflorus fermented by Lactobacillus rhamnosus is improved. After 24 hours of fermentation, the hydroxyl radical scavenging rate increased by 110%. After 48 hours of fermentation, the hydroxyl radical scavenging rate increased by 116%. After 72 hours of fermentation, the hydroxyl radical scavenging rate increased by 120%. After 96 hours of fermentation, the hydroxyl radical scavenging rate increased by 193%. This indicates that the fermentation of Lactobacillus rhamnosus can improve the hydroxyl radical scavenging ability of Platycodon grandiflorus.
7. The extract of Platycodon grandiflorus fermented by Lactobacillus rhamnosus has improved its ability to scavenge superoxide anion radicals. After 24 hours of fermentation, the superoxide anion free radical scavenging rate increased by 162%. After 48 hours of fermentation, the superoxide anion free radical scavenging rate increased by 130%. After 72 hours of fermentation, the superoxide anion free radical scavenging rate increased by 159%. After 96 hours of fermentation, the superoxide anion free radical scavenging rate increased by 161%. This indicates that the fermentation of Lactobacillus rhamnosus can improve the superoxide anion free radical scavenging ability of Platycodon grandiflorus.
8. The extract of Platycodon grandiflorus fermented by Lactobacillus rhamnosus and its total antioxidant capacity decreased. After 24 hours of fermentation, the clearance rate of superoxide anion free radicals decreased by 68%. After 48 hours of fermentation, the clearance rate of superoxide anion free radicals decreased by 74%. After 72 hours of fermentation, the clearance rate of superoxide anion free radicals decreased by 74%. After 96 hours of fermentation, the clearance rate of superoxide anion free radicals decreased by 73%.

CONCLUSIONS

1. After analyzing the active ingredients and antioxidant capacity, the extract of Platycodon grandiflorus fermented by Lactobacillus rhamnosus showed good experimental results. The content of active ingredients such as flavonoids, polyphenols, and polysaccharides in the fermentation group showed an increasing trend within 96 hours, and the content of the above active ingredients was the highest after 96 hours of fermentation. That is, after fermentation by Lactobacillus rhamnosus, the active ingredients in Platycodon grandiflorus were more easily released.

2. The content of active ingredients such as flavonoids, polyphenols, and polysaccharides in the fermented extract of Platycodon grandiflorus increases. After 24 hours of fermentation, the flavonoid content increased by 115%, the polyphenol content increased by 121%, and the polysaccharide content increased by 164%; After 48 hours of fermentation, the flavonoid content increased by 120%, the polyphenol content increased by 131%, and the polysaccharide content increased by 165%; After 72 hours of fermentation, the flavonoid content increased by 123%, the polyphenol content increased by 130%, and the polysaccharide content increased by 160%; After 96 hours of fermentation, the flavonoid content increased by 125%, the polyphenol content increased by 120%, and the polysaccharide content increased by 161%; The content of active ingredients in the control group is relatively stable.

3. The antioxidant capacity of the fermentation group was significantly improved, such as DPPH radical scavenging rate, hydroxyl radical scavenging rate, and superoxide anion radical scavenging rate. After 24 hours of fermentation, the DPPH radical scavenging rate increased by 110%, the hydroxyl radical scavenging rate increased by 110%, and the superoxide anion radical scavenging rate increased by 162%; After 48 hours of fermentation, the DPPH radical scavenging rate increased by 116%, the hydroxyl radical scavenging rate increased by 116%, and the superoxide anion radical scavenging rate increased by 130%; After 72 hours of fermentation, the DPPH radical scavenging rate increased by 120%, the hydroxyl radical scavenging rate

increased by 120%, and the superoxide anion radical scavenging rate increased by 159%; After 96 hours of fermentation, the DPPH radical scavenging rate increased by 119%, the hydroxyl radical scavenging rate increased by 193%, and the superoxide anion radical scavenging rate increased by 161%

4. There are a large number of free radicals in the human body, which can use peroxidation to damage biological macromolecules such as proteins, cell membranes, enzymes, nucleic acids, etc. They can also cause lipid peroxidation reactions in unsaturated fatty acids, leading to a decrease in enzyme activity and DNA gene mutations, which in turn damage cell function, accelerate body aging, and cause damage to the human body. Fermented Platycodon grandiflorus can be used as a basis to develop new probiotic fermented Platycodon grandiflorus raw materials with high added value and activity by clearing free radicals.

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