Development and Activity Evaluation of Antialcoholism and Liver Detox Jiaosu

Zhi-peng YANG¹, Ting WANG¹,² and Xin-li LIU¹,*

¹Shandong Provincial Key Laboratory of Microbial Engineering, Qilu University of Technology
(Shandong Academy of Sciences), Jinan 250353, P.R. China
²Fufeng Group Limited, Linyi 276600, P.R. China
*Coresponding author

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Abstract. The antialcoholism and liver detox Jiaosu selected traditional Chinese medicine with antialcoholism effect, such as pueraria lobata, hawthorn, poria cocos as raw materials. After bioenzymatic hydrolysis, Lactobacillus rhamnosus 217-8 with high glutathione production was selected as fermentation strain. After fermentation, the Jiaosu was placed in a constant temperature and humidity environment, and the contents of flavonoids, puerarin, polyphenols and antioxidant capacity of Jiaosu were determined. The results showed that the content of puerarin was increased by 7 times, flavonoids increased by 3 times, amino acids increased by 6 times, polyphenols increased by 80mg/L, the antioxidant capacity increased by 92% of antialcoholism and liver detox Jiaosu compared with the raw material without enzymatic hydrolysis and fermentation. So we can draw the conclusion that the optimization and compatibility of radix puerariae, hawthorn, poria, through enzymatic hydrolysis and microbial transformation, not only the natural active ingredients intact and release, some of the macromolecular components can be transformed into small molecular components, which are beneficial to human absorption and metabolism, but also produce active secondary metabolites at the same time, and further improve the types and functions of active components in antialcoholism and liver detox Jiaosu. So, the antialcoholism and liver detox Jiaosu developed in this paper has an important potential in accelerating the circulation of tri-carboxylic acid and promoting the decomposition of acetaldehyde, improving the concentration of ethanol and triglyceride in the blood, and improving the anti-oxidation ability of the liver tissue.

Introduction

Chinese wine culture has a long history. Moderate drinking is beneficial to people's health, and has the function of dredging blood vessels and maintaining health care. However, with the accelerating pace of modern life, due to work pressure, business entertainment and other factors lead to the number of drinking and drinking per capita in China is rising year by year. The damage caused by excessive drinking has become a common concern of the whole society. Especially long-term excessive drinking can cause chronic alcoholism, leading to serious diseases such as hepatitis, fatty liver, cirrhosis and so on, endangering human health [1,2]. In order to deal with and solve the health problems caused by excessive drinking, there are many relieving alcohol and protecting the liver products such as chemicals, traditional Chinese medicine preparations and health products have appeared in the market. Although the above products can play a role in decomposing alcohol to some extent, alleviating the symptoms of dizziness, vomiting and the like caused by high alcohol concentration, the drugs will exert metabolic burden on the liver and kidneys with varying degrees of harm while relieving the alcoholism. At the same time, there is not yet the products of relieving alcohol and protecting the liver in the market which has been completely formed from the raw materials of medicinal and edible homologues and transformed by probiotic fermentation after investigation.

In April 2014, the National Health and Planned Biological Committee of the People's Republic of China published a list of 101 kinds of raw materials for medicines and foods that are both food and
medicine in "the State Health and Family Planning Commission Affairs Office open on new food raw materials, general food and health food-related issues". The raw materials of medicinal and edible homologues not only provide the required nutrition, taste, but also produce quinones, flavonoids, tannins, terpenes, steroids and glycosides, alkaloids and other "natural products"[3]. These natural products play a similar regulatory role as drugs in regulating physiological functions, but due to the low levels of these natural products in medicinal and edible homologues materials, they are non-toxic side effects for a long time [4]. Therefore, it is of great significance to choose the raw material of medicinal and edible materials as the research object, and to develop antialcoholism and liver detox products combining with biological enzymolysis and microbial fermentation technology to alleviate the damage caused by over-drinking [5].

The main raw materials are selected and compatible with the medicine and food, such as Puerariae, Crataegus, Poria [6-8], which are developed in this paper of antialcoholism and liver detox Jiaosu. After compound enzymatic treatment, Lactobacillus rhamnosus with high glutathione production was selected for fermentation. Through the complex enzymolysis and microbial fermentation transformation, we got high quality antialcoholism and liver detox Jiaosu products which are rich in puerarin, polyphenols, flavonoids and other natural active ingredients, as well as active probiotics and beneficial metabolites [9-11]. Therefore, the antialcoholism and liver detox Jiaosu has important potentials to accelerate the tricarboxylic acid cycle and promote the decomposition of acetaldehyde, improving the concentration of ethanol and triglyceride in the blood and improve the antioxidant capacity of liver tissue [12]. It is important guiding significance to alleviate the liver damage caused by excessive drinking and the development of other enzyme products.

Material and Methods
The materials and methods are listed as follows in this study.

Strains, Reagents and Equipment
Jiaosu fermentation select Lactobacillus rhamnosus 217-8 for my laboratory to preserve probiotic strains [13]. The equipment used in the experiment has spectrometer (model LDZX-50KBS), desktop high speed centrifuge (model TG16-WS), amino acid analyzer (model L-8900), constant temperature incubator (model GNP-9160), high-performance liquid chromatography (model LC-VP). Reagent used lactic acid bacterial culture medium (MRS), anhydrous ethanol, rutin, ninhydrin, aluminum nitrate, potassium acetate, gallic acid, sodium carbonate, folin phenol, 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) and so on, the above reagents are analytical grade.

Detection of Active Lactobacillus in Antialcoholism and Liver Detox Jiaosu
The number of live bacteria in the enzyme was measured by plate count method. In clean workbench by gradient dilution of antialcoholism and liver detox Jiaosu, respectively 10⁻⁴, 10⁻⁵, 10⁻⁶ concentration gradient, with 10μl dilution in MRS agar medium, each have three parallel gradients. After the 15min was placed in the ultra clean worktable, the plates were placed in the incubator at 37°C 36h.

Determination of Puerarin Content in Antialcoholism and Liver Detox Jiaosu
Determination of puerarin in Jiaosu by high performance liquid chromatography. Chromatographic column: ODS C18, 250mm×4.6mm, 5μm; Mobilephase: methanol+36% acetic acid+ water =25+3+72; Flow: 0.6mL/min; Detection wavelength: 247nm; Injection volume: 10μl. After preparation of 100μg/mL puerarin standard with 70% methanol, 1mL, 2mL, 3mL, 4mL, 5mL, 6mL were placed in 10mL volumetric flask, and the volume was shaken with 70% methanol. The standard solution was taken and filtered through a 0.45μm filter and analyzed according to the above chromatographic conditions. With the concentration of puerarin as the abscissa and the peak area as the ordinate, the standard curve is drawn. Then take 1mL Jiaosu and the initial solution, with 70%
methanol volume in a volumetric flask of 10mL, filtered through the 0.45μm filter into the high performance liquid chromatography.

**Detection of Amino Acid content in Antialcoholism and Liver Detox Jiaosu**

The free amino acid content was measured by ninhydrin method, and then analyzed by amino acid analyzer [14,15]. Accurately absorb the amino acid standard solution 0.0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0mL, were placed in 25mL colorimetric tube and add water to the volume of 4.0mL, after adding ninhydrin solution and phosphate buffer solution 1mL, shake, water bath for 15mins, removing the rapid cooling to room temperature, adding water to the mark (25mL), then shaking. And allowed to stand for 15min. The absorbance of the remaining solutions was measured with the blank reagent as the reference solution. With the amino acid mass as the abscissa, the absorbance value is the ordinate, draw the standard curve. After the clarification of the Jiaosu solution and the initial solution of 1mL, repeat the above operations, measured its absorbance. According to the general content of free amino acids, enzyme dilution appropriate multiple by 0.45μm filter filter into the amino acid analyzer for analysis and detection.

**Detection of Plant Polyphenol Content in Antialcoholism and Liver Detox Jiaosu**

Take 1.00mg/mL gallic acid standard stock solution, prepared into 10, 20, 30, 40, 50μg/mL of the working fluid. The work of gallic acid solution and distilled water for each 1mL in the test tube, add 5.0mL 10% Folin phenol respectively, and reaction of 3-8mins after adding 7.5% Na2CO3 4mL solution, shake, place at room temperature for 60mins at the wavelength of 765nm is measured under light absorption value[16]. The standard curve is plotted with gallic acid concentration as ordinate and absorbance value as abscissa. Take 1mL to be checked, repeat the above operations, measuring its absorbance.

**Detection of Flavonoids Content in Antialcoholism and Liver Detox Jiaosu**

Accurately absorb 0.2mg/mL rutin standard solution 1mL, 2mL, 3mL, 4mL, 5mL, 6mL, were placed in 50mL volumetric flask, add 95% ethanol to the total volume of 15mL, followed by adding aluminum nitrate solution 1mL, Solution 1mL, shake, add water to the mark, shake, put it aside for 1h. To 30% ethanol solution for the blank at 415nm, measure its absorbance. Taking rutin quality as abscissa and absorbance as ordinate, the standard curve is drawn. Accurately absorb 1mL of the test solution in 50mL volumetric flask, repeat the above operation, measured its absorbance.

**Study on Antioxidant Activity of Antialcoholism and Liver Detox Jiaosu**

A sample of 100μl of a certain concentration gradient was added to the test tube and 3.9mL 0.1mmol/L DPPH ethanol solution was added. After mixing, the absorbance was measured at 37°C for 1h at 517nm. The ethanol was used as a blank control. The results are expressed as IC50 values. DPPH radical scavenging rate of 50% of the required sample concentration of IC50, DPPH free radical scavenging rate calculated according to the following formula:

\[
\text{Clearance rate(%)=[1-(As/Ac )]×100\%}
\]

Ac=Absorbance of blank control; As=Absorbance of the sample

**Results and Discussion**

**The Growth Trend of Lactobacillus Rhamnolipid 217-8 in Antialcoholism and Liver Detox Jiaosu**

It can be seen from Figure 1, the first week of fermentation stage probiotics rapid value, up to 2.12×10⁹ cfu/mL. Due to the rapid growth of strains produce large amounts of lactic acid, under acid stress and starvation, starting from the second week growth and metabolism of Lactobacillus rhamnosus inhibited the number of live bacteria began to decline, began to stabilize in eighth weeks, stable at 1.2×10⁷ cfu/mL [17]. *Lactobacillus rhamnosus* 217-8 belongs to the genus *Lactobacillus*, *L.*
rhamnosus. It is isolated from healthy intestinal flora. The strain was evaluated in vitro by the laboratory, with significant acid, bile salt and artificial gastrointestinal tolerance, and the ability to reduce cholesterol significantly and high GSH production. Therefore, the strain is used as the starting strain to microbial transformation of the antialcoholism and liver detox Jiaosu material, which has the potential to regulate the intestinal flora and promote the cell division, and can effectively remove the endotoxin produced by the decomposition of the liver cells and the stable liver Cell membrane, promote intrahepatic protein synthesis, protection of liver cells and promote the decomposition of nutrients, and thus plays a role in accelerating the body's metabolism\textsuperscript{[18]}.

![Graph](image)

Figure 1. Change curve of live bacteria number of antialcoholism and liver detox Jiaosu in three months.

**Comparison of the Content of Puerarin in Antialcoholism and Liver Detox Jiaosu**

The liquid chromatogram of puerarin standard solution is shown in Figure 2. According to the standard curve of puerarin in Figure 3, the content of Puerarin in the original solution without enzymatic hydrolysis and fermentation is 69.33mg/L, while the content of Puerarin in the antialcoholism and liver detox Jiaosu is 534.8mg/L. It can be seen by enzymatic extraction and probiotics fermentation, puerarin content increased by nearly 7 times. Puerarin has the effect of reducing blood vessel resistance, increasing brain and coronary blood flow, and has anti-free radicals and enhances SOD activity. The content of puerarin in the antialcoholism and liver detox Jiaosu was significantly improved, and the potential effect of the solution was remarkable enhanced.

![Chromatogram](image)

Figure 2. Liquid chromatogram of Puerarin standard, untreated raw solution, antialcoholism and liver detox Jiaosu.
Detection and Comparison of Amino Acids in the Antialcoholism and Liver Detox Jiaosu

The amino acid standard curve is shown in Figure 4. According to the standard curve, the amino acid content of the original solution without enzymatic hydrolysis and fermentation is $0.343 \times 10^3$ mg/L, the content of amino acids in the antialcoholism and liver detox Jiaosu is $3.96 \times 10^3$ mg/L, and the content of amino acid was nearly 10 times higher than that of the untreated original solution. After further analysis by amino acid analyzer, there are 16 kinds of amino acids in the antialcoholism and liver detox Jiaosu, and the highest content is proline. The content of essential amino acid / amino acid is 0.442, reaching the reference protein pattern of the required amino acid to total amino acid in the protein proposed by the Food and Agriculture Organization of the United Nations / World Health Organization should be above 0.40$^{[19]}$. The increase of amino acid content can accelerate the tricarboxylic acid cycle and promote the decomposition of acetaldehyde, so the antialcoholism and liver detox Jiaosu has a good relieving alcohol function.

Detection and Comparison of Polyphenol in the Antialcoholism and Liver Detox Jiaosu

The standard curve of gallic acid is shown in Figure 5. According to the standard curve, the polyphenol content of the antialcoholism and liver detox Jiaosu is $8.1 \times 10^2$ mg/L, and the polyphenol content of the original solution without enzymatic hydrolysis and fermentation is $7.3 \times 10^2$ mg/L. Polyphenols as natural plant antioxidant active ingredients, the hydrogen supply of hydroxyl radicals, superoxide anion radicals and lipid compounds and other free radicals, their own into phenolic free radicals, due to phenolic free radicals have a certain stability, thus reducing the transmission rate of the automatic oxidation chain reaction, and suppressing the occurrence of further oxidation reaction. The content of polyphenols increased by 80 mg / L after fermentation, which enhanced the antioxidant capacity of hepatocytes and reduced the damage of alcohol body.

Figure 3. Standard solution curve of Puerarin.

Figure 4. Standard solution curve of Amino acid.

Figure 5. Standard solution curve of Polyphenol.
Comparison of the Content of Flavonoids in Antialcoholism and Liver Detox Jiaosu

The standard curve of rutin is shown in Figure 6. Calculated from the standard curve that the content of flavonoids in the solution of the antialcoholism and liver detox Jiaosu is $3.78 \times 10^2$ mg/L, the original solution without enzymatic hydrolysis and fermentation had a flavonoid content of $1.24 \times 10^2$ mg/L, which can be obtained after probiotics fermentation of flavonoids increased by about 2 times. The increased content of flavonoid can be significantly reduced the MDA content, triglyceride (TG) content, increased glutathione (GSH) content in liver tissue, while improving the liver tissue superoxide dismutase activity, inhibiting the release of free radical, thereby reducing liver tissue pathological damage.

Comparison of Antioxidant Ability of the Antialcoholism and Liver Detox Jiaosu

Alcohol on liver cell toxicity can affect the metabolism of liver cells, liver cell membrane surface lipid composition over-oxidation, destruction of liver cell membrane. And excessive drinking after the body of superoxide dismutase, malondialdehyde, glutathione peroxidase, glutathione significantly changed, the body produces a large number of oxygen free radicals to enhance the oxidation reaction, resulting in a large number of free radicals, which produces great poisonous to the organization of body. Therefore, it is of great significance to study the antioxidant capacity of the antialcoholism and liver detox Jiaosu to protect liver. Pueraiae, Poria and other homology of medicine and food contain a large number of natural antioxidants polyphenols and flavonoids, to remove free radicals in the human body to protect the liver has a great effect, combined with lactic acid bacteria for fermentation, improved the body's antioxidant activity, thus effectively remove the free radicals in the human body.[20]. Antioxidation of the antialcoholism and liver detox Jiaosu was studied by DPPH antioxidant test to simulate the scavenging of free radicals.
The free radical scavenging rate curve of the solution of the antialcoholism and liver detox Jiaosu was calculated by DPPH antioxidant experiment as shown in Figure 7, and the IC50 value is 0.088. The free radical scavenging curve of the original solution without enzymatic hydrolysis and fermentation is shown in Figure 8 with IC50 value of 0.1693. By comparison, it can be found that the free radical scavenging rate of the liver and liver defense enzymes has increased by 92%, and has higher antioxidant capacity.

Summary

In this paper, the development of the antialcoholism and liver detox Jiaosu from the raw material complex, composite enzymatic hydrolysis process optimization, microbial fermentation, thermostatic ripening four aspects of experimental research, from the following four angles to ease drunkeness, the role of liver protection: (1) Lactic acid in the stomach to form a protective layer to protect the gastric mucosa at the same time to prevent alcohol and absorption, and puerarin can be used as gastrointestinal absorption inhibitors, which together inhibits alcohol gastrointestinal absorption. (2) Flavonoids can significantly induces the activity of P450 enzyme in the liver and enhances the elimination rate of alcohol and its metabolites. (3) The abundant amino acid can regulate the metabolism disorder caused by alcohol. (4) Polyphenols improves the body's antioxidant capacity, the effective removal of alcohol metabolism free radicals and eases the liver cell membrane lipid composition of the excessive oxidation. The natural active ingredients such as puerarin, amino acid and flavonoids in the solution were significantly improved compared with those before fermentation.

In this paper, the development of a highly biologically active solution of the antialcoholism and liver detox Jiaosu products for research purposes, by comparing Jiaosu with puerarin, total flavonoids, polyphenols, amino acids and antioxidant capacity in the aqueous solution of water extracted without any treatment. It was found that after enzymatic hydrolysis and probiotic Lactobacillus rhamnosus 217-8, fermentation of the Jiaosu by biological extraction and microbial fermentation transformation, the raw materials can be relieving alcohol effect of natural active ingredients are retained and released at the same time, produce strains of active metabolites. The antialcoholism and liver detox Jiaosu can accelerate the tricarboxylic acid cycle and promote the decomposition of acetaldehyde, improving the
antioxidant capacity of liver tissue. It can effectively prevents and alleviates the liver damage caused by excessive drinking, and protects the health of people.

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