Taste Correction and Safety Evaluation of Rhodiola Extraction

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Abstract. Objective To explore tannins removal of Rhodiola extraction and evaluate its safety. Method: Content of Tannins was treated with cheese, tannins removal was used by chitosan embedding, safety evaluation was tested by acute toxicity. The pharmacodynamics were detected by in vivo and in vitro antioxidant methods. Results: 20g/L Rhodiola extraction added 1g chitosan under 50°C embedding 2h, tannis was removed effectively and taste improved greatly. The results of acute toxicity tests in mice showed that the daily dose of 29.73 g was safe on the basis of 70kg adult weight. Antioxidant activity in vitro was evaluated by DPPH, super-oxide anion and hydroxyl radical scavenging assays showed at concentration of 0.1% indicated better results, while results in vivo showed at concentration of 1.2% decreased p53, p21 gene expression. Conclusion: A reasonable process for removing tannins from were determined Rhodiola extraction and taste of Rhodiola extraction is safe under the removal extraction technique. Results of antioxidant proved that Rhodiola extraction is effective.

1. Introduction
Rhodiola is a plant of Rhodiola family, the current Pharmacopoeia variety is Rhodiola crenulata. It is widely believed that it has the effects of anti - ischemia, anti - hypoxia, anti - fatigue and improving athletic ability[1]. Rhodiola mainly contains rhodiola glycosides, tannins and polysaccharides[2-3]. The tannins have seriously affected the clarity and taste of Rhodiola rosea extract preparations, especially its strong bitter taste, which has affected the development and utilization of Rhodiola rosea in the health products market. In order to correct its bitter taste, it is necessary to remove the enamel components as much as possible. At present, the methods for removing tannin in China are generally classified into alkaline alcohol precipitation method, dialysis method, gelatin precipitation method and chitosan precipitation method[4-6]. This study investigated the effects of different chitosan dosage, agitation temperature and embedding time on the taste of rhodiola rosea. On this basis, the safety and pharmacodynamics of the modified rhodiola rosea extract were evaluated to provide theoretical basis for the further development and application of the health food with Rhodiola rosea as the main component.

2. Materials and Methods
2.1 Materials
Rhodiola (Chinese medicinal materials wholesale market purchased), approved by Professor Qin
Jiamei from the School of Life Sciences of Tonghua Normal University as *Rhodiola crenulata*; mice (SPF kunming mice, purchased from changchun gaoxin animal center, male and female, healthy and age-appropriate); zebrafish is provided by Metabolism and Aging Laboratory of Tonghua Normal University. All the drugs used in the experiment were analytically pure.

2.2 Methods

2.2.1 Determination of tannin content. Operate in accordance with Appendix XB of the 2015 Edition (Part 1) of the Pharmacopoeia of the People's Republic of China.

2.2.2 Method for the removal of *Rhodiola* tannin. The dried *Rhodiola* was cut into pieces and crushed through a 30-mesh sieve. 20g rhodiola powder was added to 400ml distilled water and soaked for 24h. The mixture was heated and extracted in a water bath at 85°C for 1.5 h, suction filtered, and the filtrate was removed, the remaining part was adjusted to a volume of 1000 ml to prepare *Rhodiola rosea* extract. Divided the *Rhodiola* extract into 4 groups, investigate different doses of chitosan (0.5g, 1.0g, 1.5g, 2.0g), different mixing times (0.5h, 1.0h, 1.5h, 2.0h) and stirring temperature (30°C, 40°C, 50°C, 60°C) effect on its tannin content. Each group of experiments was repeated 3 times to determine the average.

2.2.3 Modified Rhodiola Extract Safety Test. Maximum toxicity test for acute toxicity test: 20 mice, 10 in each group, were randomly divided into the drug-administered group and the control group. Administration group: given the maximum volume (0.4 mL/kg) available for this product (the average weight of the mice was 20 g, and the maximum dosage was about 9.8g/kg). One night before the administration, the mice were fasted for 12 hours, and the next day, the administration was started. The administration interval was 3 hours. During the interval, the mice were fasted but allowed to drink, and the drug was administered continuously for 7 times. Control group: The same amount of distilled water was administered, and the time, duration, recovery period, number of deaths, etc. of the mice were recorded at 12 h after administration.

2.2.4 Study on pharmacodynamics of *Rhodiola* extract. Experimental study on antioxidant activity of *Rhodiola* extract

The 0.005%-0.1% *Rhodiola* solution was prepared separately. The superoxide anion radical scavenging rate was determined by pyrogallol auto-oxidation method, and the hydroxyl radical scavenging rate was determined by FeSO4 salicylic acid method.

Experimental study on the antioxidant activity of *Rhodiola* extract

The control group (CK), low concentration group (0.6%), medium concentration group (1.2%), and high concentration group (1.8%) were prepared respectively to treat zebrafish embryonic cells. The culture medium was changed once every day, and the cells were continuously treated for 3 days. Samples were collected for gene expression analysis.

2.2.5 Statistical analysis. Data of experimental results were expressed as $\bar{x} \pm s$, and SPSS16.0 software was used for one-way analysis of variance, and $p<0.05$ was considered to be statistically significant.

3. Results and Analysis

3.1. Effect of Different Chitosan Dosage on Tannin Removal

Tannins are widely found in the plant kingdom, and more than 70% of Chinese herbal plants contain tannins. In the extraction process of Chinese herbal medicines, especially in the extraction process of the solution, the tannin is often removed as an impurity. Under the experimental conditions, the effects of different chitosan on tannin removal were shown in figure 1. As can be seen from figure 1, with the increase of chitosan dosage, the change trend of tannin content first decreases, then increases and then
decreases. When chitosan content reached 1.0g, the tannin content was the lowest, and then increased with the increase of chitosan content.

![Figure 1](attachment://image1.png)

**Figure 1.** Effect of different chitosan dosage on tannin removal

3.2. Effects of Different Agitation Time on Tannin Removal

Different agitation times have a greater impact on the embedding of tannins, as shown in figure 2. It can be seen from the figure that the maximum tannin content was 0.693mg/ml when the agitation time was 1.5h. When the embedding time reached 2h, the tannin content reached a low value of 0.425 mg/ml. Subsequent experiments showed that the content of tannin remained at about 0.425 mg/ml as the agitation time increased.

![Figure 2](attachment://image2.png)

**Figure 2.** Effects of different agitation time on tannin removal

3.3. Effects of Different Agitation Temperatures on Tannin Removal

After adding the same chitosan, the influence of different stirring temperatures on tannin content was shown in figure 3. It can be seen from the figure that in the temperature range of 30°C to 60°C, the content of tannin increases with the increase of temperature. When the temperature reached 50°C, the content of tannin was the lowest, and then the content of tannin showed an increasing trend with the increase of temperature.
3.4. Effect of Modified Rhodiola Extract on Toxicity and Hypoxia Tolerance in Mice

The product shall be given in the maximum available volume (0.4ml/kg). (The average weight of the mice was 20g, and the maximum dosage was about 9.8g/kg). After continuous administration for 7 days (Table 1), no abnormality or death was found in mice after intragastric administration, indicating that the modified Rhodiola extract used in this study was actually non-toxic. Subsequently, the effects of different concentrations of Rhodiola extract on survival time of mice under normal pressure were investigated (Table 2). The results showed that the survival time of mice could be prolonged by different concentrations of Rhodiola extract. Compared with the control group, the survival time of mice in the high-dose group reached a significant level (p<0.05), indicating that Rhodiola extract could improve the anti-hypoxia ability of mice.

| Times of administration | The first administration | The second administration | The third administration | The fourth administration | The fifth administration | The sixth administration | The seventh administration |
|-------------------------|-------------------------|--------------------------|-------------------------|--------------------------|-------------------------|--------------------------|---------------------------|
| Dosage (mL)             | 0.4                     | 0.4                      | 0.4                     | 0.4                      | 0.4                     | 0.4                      | 0.4                       |
| Deaths                  | None                    | None                     | None                    | None                     | None                    | None                     | None                      |

Table 2. Effects of different concentrations of Rhodiola extract on hypoxia survival time of mice under normal pressure ($\bar{x} \pm s$).

| Groups                  | Dosage (g/kg) | Survival time (min) |
|-------------------------|---------------|---------------------|
| Control group           |               | 28.42 ±6.25         |
| *Rhodiola extract group*|               |                     |
| Low dose group          |               | 26.76±8.35          |
| Medium dose group       |               | 27.08±6.56          |
| High dose group         |               | 30.63±5.87*         |

*The significance level was 0.05

3.5. Experimental Study on Antioxidant Activity In Vitro of the Modified Rhodiola Oral Solution

Different concentrations of modified Rhodiola have certain scavenging effects on DPPH, superoxide anion radicals and hydroxyl radicals, as shown in Table 3. It can be seen from Table3 that the removal rate of superoxide anion reached 96.33% when the concentration of modified Rhodiola oral solution was 0.1%. At the concentration of 0.05%, the DPPH clearance rate was the highest at 89.01%. Although this experiment has removed the tannin of Rhodiola, it still contained a certain amount of...
tannin, so the scavenging rate of hydroxyl radical was relatively low.

**Table 3. Effects of *Rhodiola* Oral Liquid on Hydroxyl Radical, Superoxide Anion Radical and DPPH Clearance.**

| Rhodiola oral liquid concentration | Hydroxyl radical (%) | Superoxide anion free radical(%) | DPPH(%) |
|-----------------------------------|----------------------|----------------------------------|---------|
| 0.1%                              | 10.06                | 96.33                            | 83.67   |
| 0.05%                             | 9.52                 | 91.33                            | 89.01   |
| 0.01%                             | 4.79                 | 91.0                             | 77.67   |
| 0.005%                            | 1.06                 | 87.65                            | 51.33   |

3.6. Experimental study on antioxidation of modified *Rhodiola* oral liquid in vivo

In combination with the in vitro antioxidant experiment, the zebrafish embryo cells developed for 8h were treated by low-concentration group, medium-concentration group and high-concentration group of *Rhodiola*. And after 24 hours of drug treatment, the effects of each treatment on the survival rate and deformity rate of zebrafish were valued(Figure 4). After 3 days of treatment, the effects of aging-related gene expression were examined(Table 4). The results showed that the treatment of medium concentration drug had little effect on the development of zebrafish, but the treatment of high concentration group affected the survival and development of zebrafish, and the mortality and deformity rate were higher. In addition, low concentration treatment can significantly reduce the expression of p53 and p21 genes. Compared with the control group, there was no significant difference in the expression levels of p53 and mdm2. High concentration group treatment increased the expression of p53 and p21 genes.

**Figure 4.** Effect of modified Rhodiola Oral Liquid on Survival Rate and Deformity Rate of Zebrafish Embryos(Figure A normal developing zebrafish, B deformed zebrafish)(The data in the figure is the standard deviation of 30 zebrafish embryos).

**Table 4. Effect of the treatment of modified *Rhodiola* Oral Liquid on the expression of aging-related genes in zebrafish (The data in the table is the standard deviation of 30 zebrafish embryos).**

| gene | control group | low concentration | medium concentration | high concentration |
|------|---------------|-------------------|----------------------|--------------------|
| mdm2 | 1.0012±0.0599 | 1.0000±0.0080     | 1.0915±0.1706        | 1.0057±0.0061      |
| p53  | 1.0008±0.0492 | 0.9377±0.0110     | 1.0118±0.0736        | 1.7252±0.0206      |
| p21  | 1.5301±0.0370 | 1.4242±0.0262     | 1.4579±0.0273        | 1.8095±0.0473      |
4. Discussion

In this paper, the single factor experiment method was used to investigate the addition amount, agitation time and agitation temperature of chitosan. The final determined rationality of Rhodiola extract taste correction is that the dried Rhodiola was cut into pieces and crushed through a 30-mesh sieve. 20g Rhodiola powder was added to 400ml distilled water and soaked for 24h. The mixture was heated and extracted in a water bath at 85℃ for 1.5h, suction filtered, and the filtrate was removed, the remaining part was adjusted to a volume of 1000ml to prepare Rhodiola rosea extract. Take 100 ml of Rhodiola extract, add 1.0 g chitosan and stir at 500/min in an electromagnetic heating stirrer, heat at 50℃ for 3h, filter and dilute to 100 ml. This study laid a foundation for the orthogonal test in the future to optimize the taste correction process of Rhodiola extract.

At the same time, according to the standard requirements of the Food Safety Toxicology Evaluation Procedure, the safety test and hypoxic tolerance experiment were conducted on the modified Rhodiola extract. Under the experimental conditions, with the increase of the concentration of Rhodiola rosea extract, the hypoxia tolerance of the experimental mice increased accordingly. According to the adult body weight of 70 kg, the daily dose was 29.73g, with no adverse effects. Therefore, the modified Rhodiola extract used in this study was actually non-toxic.

The main reason for human aging is that with the increase of age, the ability to scavenge free radicals in the body decreases, and free radicals have become the root cause of 85% of the human body's diseases[10]. Looking for low-cost and suitable antioxidants for the human body has been a hot topic of scientific research. Compared with synthetic antioxidants, natural antioxidants have more extensive development and utilization prospects[11]. Higher organisms are basically developed from single cell fertilized eggs. Many scholars believe that life is most sensitive to exogenous chemicals in the embryonic period, so it is of great significance to monitor them. The zebrafish can be observed directly under microscope because of its in vitro fertilization, in vitro development and transparent embryo body. In addition to being a tumor suppressor gene, p53 also has many important functions, including cell apoptosis and cell senescence. When subjected to stress signals such as DNA damage, hypoxia and radiation, p53 exerts its transcriptional activation function, regulates the transcriptional expression of a series of target genes, and then causes stress reactions such as DNA damage repair, cell cycle arrest and apoptosis[12]. P21 is activated by p53 at the transcriptional level and mainly mediates aging caused by telomere dependence and various emergency conditions such as DNA damage[13]. Mdm2 is currently the most important intracellular negative regulator of p53. Mdm2 contains a p53 gene binding site that binds to p53 to form a complex that inhibits the transcriptional activity of p53. Overexpression of mdm2 can block the trans-activation mediated by p53, resulting in loss of p53 function, gene instability and cell proliferation. DNA damage leads to mdm2 inactivation and elevated p53 levels[14]. Based on the above analysis, it can be seen that the modified Rhodiola Oral Liquid has certain antioxidant activity of scavenging free radicals in vitro. And this effect may be mediated by the p53 signal transduction pathway, which provides experimental basis for further research and development of Rhodiola natural antioxidant drugs.

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