MINISTRY OF EDUCATION AND SCIENCE OF UKRAINE
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 Enzymatic Extraction of Tectorigenin from *Iridis Tectori Rhizoma***

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

Completed: student of group BEBT-20
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ASSIGNMENTS
FOR THE QUALIFICATION THESIS
Shao Zhihao

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Scientific supervisor Olga Andreyeva, Dr. Sc., Prof.

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I am familiar with the task:

Student _____ Shao ZHIHAO

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SUMMARY

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Antibiotics are gradually abused in the animal feed industry, which will cause the decline of self-immunity and the emergence of drug-resistant bacteria. When people eat meat and egg products, antibiotics will be enriched in the human body, leading to adverse effects. To this end, the state limits the production of antibiotic feed and promotes the use of Chinese herbs as the best alternative to antibiotics. Chinese herbs themselves do not produce harmful substances and are a kind of green resources, Chinese herbs play a role because of the natural active ingredients in them. Enzymatic method can specifically degrade plant cell wall and release natural active ingredients through the barrier of cell wall.

In this paper, the enzyme method was used to extract tectorigenin from Iridis Tectori Rhizoma. Acid protease, acid cellulase, pectinase (II) and xylanase were selected to act on Iridis Tectori Rhizoma respectively. Using the standards of two substances, the standard curves of tectoridin and tectorigenin were determined by HPLC. The samples were extracted by enzymolysis. The extraction amounts of tectorigenin of acid protease, acid cellulase, xylanase and pectinase (II) were 47.7 ug/g, 89.8 ug/g, 60.0 ug/g and 50.0 ug/g respectively, and the extraction amount of acid cellulase was the highest. In addition, the experiment of ultrasonic-assisted enzymolysis was carried out, and the extraction amount of acid cellulase group was increased by 91.8% compared with that of water extraction group. In tectoridin extraction experiment, the extraction effect of pectinase (II) was the best, reaching 7.57 mg/g, which indicated that pectinase (II) had a significant effect on cell wall hydrolysis.

Key Words: Enzymatic Extraction, Iridis Tectori Rhizoma, Tectorigenin, Tectoridin, Acid protease, Acid cellulase, xylanase, pectinase*(II)*

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INTRODUCTION

The Chinese herbal medicine Iridis Tectori Rhizoma, which can be used to prevent and cure sore throat, cough and asthma, was included in the Pharmacopoeia of the People's Republic of China in 2005. Tectorigenin is the most effective structural substance of Iridis Tectori Rhizoma, its absorption and efficacy are significantly higher than Iridis Tectori Rhizoma itself, and its toxic side effects on the recipient are significantly lower. Therefore, it is also hoped that the conversion rate of Iridis Tectori Rhizoma to tectorigenin can be improved through the action of enzymes, and the efficacy of the final product can be enhanced.

In this paper, the enzymolysis of acid protease, acid cellulase, xylanase and pectinase (II) were used to compare the production of tectorigenin. The general procedure followed in this experiment is: the enzymolysis solution sample is made first, and there will be a blank control group, which is the first treatment. The second treatment will be different, some samples through ultrasonic treatment for 5 minutes, ultrasonic power 360 W, ultrasonic temperature 25 ℃. Some samples will be extracted by water at a temperature of 50 ° C for 4 hours. Finally, it is centrifuged by centrifuge, considering that there will be partial precipitation after the sample is left standing. The correlation peak graph of each group was obtained by high performance liquid phase spectrograph. Then the extraction amount was calculated by the standard equation to evaluate the effect of each enzyme.

The relevance of the topic is an interdisciplinary study, with theoretical significance. The obtained enzymatic hydrolysis process can be applied to industrial production, improve production efficiency, and has certain practical significance.

The purpose of the Chinese medicinal material used in this paper is Iridis Tectori Rhizoma. The yield of natural active substance tectorigenin in Iridis Tectori Rhizoma was investigated by enzymolysis effect of four enzymes. These four enzymes are acid protease, acid cellulase, xylanase and pectinase (II). After the sample was made, the peak area map was obtained through high performance liquid

chromatography, and then the yield of tectorigenin was calculated, so as to screen out the enzyme with better effect.

The objectives of the study on Enzymatic Extraction of Tectorigenin from Iridis Tectori Rhizoma.

The object of the study the enzymolysis effect of four enzymes was evaluated. The substrate is Iridis Tectori Rhizoma, and the enzymatic solution containing tectorigenin can be obtained.

The subject of the effects of the four enzymes were evaluated by HPLC for the content of tectorigenin in the enzymolysis solution.

Research methods

1. Preparation of pH buffer

2. Preparation of enzymolysis sample:

Parallel experiment and control group were set up. It is divided into the first treatment and the second treatment, including ultrasound and water extraction.

3. Preparation of standard samples:

Draw standard curves.

4. Concentration of enzymolysis solution sample:

Using rotary evaporation instrument.

5. Detection of total samples:

Peak map can be obtained by HPLC. The mobile phase is prepared before testing and all samples are centrifuged and filtered.

The practical significance of the results obtained is to provide theoretical research and guidance for improving production efficiency, simplifying production process and reducing raw material waste in industrial production of Tectorigenin.

CHAPTER 1
LITERATURE REVIEW

1.1 Research Background

1.1.1 Application of antibiotics in feed industry

(1) History of antibiotics

Antibiotic is produced by some microorganisms (bacteria, fungi, etc.) or plants and animals that can inhibit the proliferation of microorganisms and other cells[1], common penicillin, tetracycline, rifampin and so on. The mechanism of action of antibiotics reflects the principle of "antagonism", which can be divided into four types: affecting the replication and transcription of DNA or RNA (by blocking the synthesis of folic acid, also known as coenzyme F, and participating in the generation of nucleotides as a carbon unit carrier), inhibiting the generation of proteins, affecting the permeability of cell membranes, and preventing the formation of cell walls [2].

In 1928, Alexander Fleming discovered the first antibiotic, penicillin, which promoted the modern era of antibiotic research[3]. During this period, infectious diseases were prevalent, so the research and development of related drugs was an urgent matter. The discovery of sulfonamides in 1936 and penicillin in 1942 both opened the way for antibiotic drug therapy and marked a major turning point in medicine[4]. Many have been cured by antibiotic drugs. However, with the rapid development of science and technology and the highly upgraded production technology, the manufacturing cost of antibiotics has dropped significantly, coupled with people's incomplete and unscientific cognition of antibiotics, the lack of formal purchase process and the untimely control by the state have gradually led to the abuse and misuse of antibiotics[5].

(2) Abuse of antibiotic feed

First, the long-term consumption of feed containing antibiotics in livestock and poultry will cause the residual amount of antibiotics in the body to increase. On the one hand, drug residues in the body may induce the generation of durable drug bacteria; on the other hand, residual antibiotics reduce the immune effect of immune vaccines,

laying a hidden danger for controlling the occurrence of diseases[6]. Second, cause double infection: double infection, also known as repeated infection, refers to the long-term use of antibiotics, can make sensitive bacteria inhibited, and some insensitive bacteria (such as fungi, etc.) take the opportunity to grow and reproduce, resulting in new infections. Because the use of antibiotics can cause changes in the flora, the microorganisms resistant to such antibiotics can cause new infections[7]. Third, the quality of livestock and poultry products is affected: the irregular use of antibiotics is likely to cause livestock and poultry to be in a "sub-health" state, resulting in antibiotic residues or excessive levels of livestock and poultry products (such as meat, eggs and milk products), which may reduce the shelf life of livestock and poultry products[8]. In addition, when people eat livestock and poultry products, antibiotics collect in the human body through biological enrichment, causing adverse effects.

(3) Antibiotic feed production is restricted

China produces about 210,000 tons of antibiotic raw materials every year, of which 97,000 tons are used in animal husbandry, accounting for 46.1% of the total annual output[9]. The abuse of antibiotics in animal husbandry in our country has been very obvious, therefore, the state has begun to introduce various policies to restrict the abuse of antibiotics. In 2016, the National Action Plan for the Containment of Bacterial Resistance (2016-2020)[10], jointly published by 14 departments of the State, advocated to increase the research and development of new anti-infective drugs, and strengthen the production, circulation and evaluation of antibacterial drugs. In 2021, the Ministry of Agriculture and Rural Affairs of China issued the National Action Plan for Reducing the Use of Veterinary Antimicrobial Drugs (2021-2025)[11]. This program proposes solutions to the existing problems such as drug resistance of animal-derived bacteria and excessive drug surplus. One of them is to develop alternatives to antibiotics, vigorously promote the development of veterinary Chinese medicine industry, and further develop traditional veterinary Chinese medicine. Some social figures and research experts also advocate the idea of replacing veterinary antibiotics: Representative Lu Qingguo proposed to vigorously develop plant-derived feed additives[12]. As early as in ancient times, it was recorded in Shennong's Herbal Classic that "the tung leaves feed pigs, which are three times fatter and

easy to raise" [13]. It shows that ancient people have already begun to pay attention to the use of plant-based feed additives. In modern times, Chinese herbal feed additives have broad prospects and great room for development and progress. In this "prohibition" background, it is pointed to a bright way, that is, the use of Chinese herbs to replace antibiotics in animal feed.

1.1.2 The choice of antibiotic substitutes - Chinese herbal medicine

Chinese herbs are mainly composed of three parts: plant (mainly including the three vegetative organs of plants: roots, stems, leaves, and one of the reproductive organs: fruit), animal (mainly epidermis, bone, and internal organs), and mineral[14]. Plants account for the majority of Chinese herbal medicine. As the birthplace of Chinese herbal medicine, China has more than 10,000 kinds of plant medicines (representative: ginseng, wolfberry, Chuanshigan, Astragalus, etc.), which is ahead of the whole world, among which more than 5,000 kinds of Chinese herbal medicines rich in antibacterial ingredients[15]. At the same time, Chinese medicine, which uses Chinese herbs to treat patients, was also born. Since ancient times, there have been many great Chinese herbal medicines works spread in the world, including "Compendium of Materia Medica", "Shennong Materia Medica" and so on, which all reflect the ancient people's attention to the understanding of Chinese herbal medicine.

The reason why Chinese herbs can replace antibiotics is that they have no side effects, do not make bacteria resistant, and have less residue, which is very important. Because Chinese herbs are green, safe and natural. Chinese herbal medicine itself has a variety of natural active ingredients. Some of these active ingredients have a synergistic effect, and some will generate new rare substances under specific conditions, further improve the efficacy, but also make the pharmacological functions of Chinese herbs more diversified. With various types of Chinese herbs, abundant systems, convenient access and affordable prices, artificial cultivation has been carried out in many places [16].

1.1.3 Introduction of Chinese herbal active substances

The natural active ingredients of Chinese herbs mainly include four types: phenols, flavonoids, alkaloids and terpenoids[17]. The most important function of phenols is reflected in its antioxidant capacity[18], and it also has a certain lipid reduction effect. The representative substances include cocoa polyphenols, tea polyphenols, and so on. Flavonoids also have antioxidant capacity, in addition to the treatment of cardiovascular disease, liver protection and detoxification functions. In animal husbandry, flavonoids can be used as estrogen to enhance animal production performance and improve animal immunity[19]. Representative substances are apigenin, hesperidin and so on. The significant function of alkaloids is antibacterial[20], and the representative substances are colchicine, morphine and so on. The last category is terpenoids, which are used in spices. Some hormones also belong to terpenes, representing substances such as gibberellin and so on.

1.1.4 Extraction method of the active substance of Chinese herbal medicine

(1) Traditional extraction method

The traditional extraction methods include decocting, soaking and extraction, distillation, etc. Decocting method: Suitable for hard Chinese herbs, put the herbs into a container, add water to boil and continue for a period of time, in which the active substances will dissolve into the water. Soaking extraction method: Put the medicinal materials into a specific solvent, which can be divided into water extraction, alcohol extraction and so on. Distillation method: suitable for extracting volatile components, heating medicinal materials, generating steam, and then condensing into liquid. The extraction rate of these methods is low, the impurities are more, and a lot of drug residue is produced.

(2) Modern extraction methods

Modern, there are more efficient extraction methods: ultrasonic extraction (extraction) method: the principle is that ultrasonic has three effects (cavitation, heat, mechanical), but also shoulder the three advantages of short time, high efficiency and low temperature. Song Ruixiang et al. used ultrasonic method to extract polysaccharide

from Artemisia salicyphylla, and concluded that ultrasonic extraction has the characteristics of high efficiency, fast speed and no pollution[21]. Microwave extraction (extraction) method: The principle is that under the action of microwave field, the dielectric constant of each component inside the substance is different, and then the microwave absorption capacity of each component will be different, which will be selectively heated, so that the target component can be separated. It has the advantages of high efficiency and pure product. Yuan Juli et al. used microwave to extract polysaccharides from Astragalus membranaceus. The optimal process was as follows: solid-liquid ratio 1:15, water extraction for 15 min, microwave power 231 W, polysaccharide extraction rate of 3.52%, and extraction efficiency reached the maximum[22]. Semi-bionic extraction method (SBE) : The principle is to simulate oral administration, the drug will be absorbed through the digestive tract, using a fixed pH condition to extract the active ingredient. Fan Baorui et al. adopted semi-bionic method to extract ursolic acid and oleanolic acid from Prunella, and found that the extraction efficiency was higher than that of traditional alcohol extraction methods[23]. Supercritical fluid extraction method: physical extraction method, using the fluid in a special state as the extraction agent. The principle is to separate and extract each component by changing the density of the fluid according to the characteristics of its dissolution capacity and its own density. Qian Miaoyu et al proposed the advantages of supercritical fluid extraction in the extraction and processing of fish oil[24]. Biological enzyme extraction method: Because plant cells have thick cell walls, and because the natural active ingredients of plants are mostly stored in the cytoplasm, it is necessary to break the barrier of the cell wall. Plant cell walls are mainly composed of three substances: cellulose, hemicellulose, and pectin. Biological enzyme method uses the specificity and specificity of various enzymes to act on specific components in plant cell wall, so as to degrade the cell wall and make the natural active components flow out. Commonly used enzymes are cellulase, pectinase, xylanase and complex enzyme combinations, etc. Since the vast majority of enzymes are proteins, the factors affecting the activity of proteins should be considered in the enzymolysis experiment: enzymolysis temperature, enzymolysis pH, substrate and product content, and so on.

1.2 Research status of Iridis Tectori Rhizoma and Tectorigenin

1.2.1 Introduction to enzymatic extraction of Tectorigenin from Iridis Tectori Rhizoma

(1) Introduction of Chinese herbal medicine Iridis Tectori Rhizoma

Iridis Tectori Rhizoma, a native Chinese medicine, is the dry rhizome of Iris tectorum Maxim, an irregularly shaped or conical plant, slightly flat, with branches, grayish-yellow brown or brown surface, with ring lines and longitudinal channels. It can be used to prevent and cure sore throat, cough, asthma and other symptoms, mainly distributed in southwest China, East China and South China. Chuan Shegan was included in the Pharmacopoeia of the People's Republic of China in 2005 [25].

(2) Introduction of two types of natural active substances

Tectoridin, molecular formula: $C_{22}H_{22}O_{11}$, molecular weight: 462.4. Physical properties are colorless or slightly yellow crystal or powder substance, soluble in water and polar organic solvents. Tectorigenin can be isolated from tectoridin.

Tectorigenin, chemical name 5,7,4'-trihydroxy-6-methoxy-isoflavone, molecular formula: $C_{16}H_{12}O_6$, molecular weight: 300.26, is a natural pigment, can also be called vitamin P, showing light yellow, belongs to isoflavone compounds, natural active ingredients in Iridis Tectori Rhizoma.

(3) Enzymatic hydrolysis principle

The surface of Chinese medicinal materials is hard and dense, which affects the release of the contained fluid. Through the compound action of cellulase, xylanase, pectinase and even protease, the barrier of the release of components in medicinal materials is reduced, and the extraction efficiency is improved. In addition, tectorigenin is the most effective structural substance of Iridis Tectori Rhizoma, its absorption and efficacy are significantly higher than Iridis Tectori Rhizoma itself, and its toxic side effects on the recipient are significantly lower. Therefore, it is also hoped that the conversion rate of Iridis Tectori Rhizoma to tectorigenin can be improved through the action of enzymes (such as glucose oxidase and mannanase), and the efficacy of the final product can be enhanced.

1.2.2 Research status at home and abroad

(1) Domestic research status

In recent 10 years, people have used a number of isolation and detection methods for tectorigenin and its analogues: including HPLC, RP-HPLC, high performance capillary electrophoresis ultraviolet detection, HPLC-DAD and so on. Nowadays, biological enzyme extraction of natural active ingredients of Chinese herbal medicine has emerged: including single enzyme method, compound enzyme method, multi-method collaborative extraction (ultrasonic assisted enzyme method, micro-blog assisted compound enzyme method, etc.). At present, although there are relatively few studies on the enzymatic extraction of tectorigenin, with the increase of studies on the physiological activity of tectorigenin and its analogistic, coupled with the application of separation methods and enzyme method above, we can have a reasonable prospect of this. In the future, there will be researches on the extraction of tectorigenin by enzyme method or enzyme method in collaboration with other methods. It covers all aspects from extraction, separation, detection and application. The following introduces some domestic research status:

The first was a patent[26] for a method of extracting tectorigenin from Iridis Tectori Rhizoma. The extracts were obtained by water extraction, adsorption, elution, concentration and drying. Then the extract was placed in the buffer solution. At this time, enzymatic method was used and β-glycosidase was used for enzymatic hydrolysis to obtain the enzymatic solution. After filtration to obtain the first filter cake. Then it is dissolved with ethanol reflux, and the second filter cake is obtained after filtration. Then dissolved with acetone and water reflux, the product of tectorigenin can be obtained after recrystallization. The patented method has the advantages of high yield, high purity and simple process. In this method, the enzyme method is used to extract, and after three times of filtration, the product of tectorigenin is obtained.

Li Lin et al.[27] used the ionic liquid extraction method to extract the tectorigenin homologous with similar structure from the medicinal stem. Among them, 1-ethyl-3-methylimidazole tetrafluoroborate [EMIM][BF4] was used as the ionic liquid, and four kinds of iris lutein homolog were extracted, namely, tectorigenin, iristectorigenin A,

irigenin and irisflorentin. The optimal extraction conditions were as follows: ionic liquid 1-ethyl-3-methylimidazole tetrafluoroborate [EMIM][BF4] with a concentration of 1.0 mol/, solid-liquid ratio of 1:30 (mL/g), ultrasonic assisted extraction time of 30min. In general, the process has the advantages of simple operation, stable extract and short energy consumption, and can be used as one of the effective methods to extract the same series of tectorigenin with similar structure from medicinal herbs. At the same time, on the basis of this method, the crude body fluid components extracted by ionic liquid were separated by high performance liquid chromatography (HPLC), and the structure of each component in the extract was identified by LC-MS technology, so that more specific information of each component could be obtained.

Liu Jing et al.[28]used high performance liquid chromatography (HPLC) to measure the Pak value of tectorigenin and pika of iristectorigenin A as 6.49 and 6.51 respectively under the setting conditions of acetonitrile-various pH phosphate buffers as mobile phase and wavelength of 265nm. Cheng Xuejia[29] used the fractional model experiment of asthma to prove that tectorigenin may alleviate oxidative stress by regulating Sirt1 (silence information regulator, a kind of histone deacetylase) to realize the inhibition of airway inflammation in asthma. We can further enrich our basic understanding of tectorigenin through these previous studies, and provide convenience for subsequent relevant studies on tectorigenin.

(2) Foreign research status

Jinmen Kim and Yejin Lee[30] et al extracted anthocyanins from the fruit dregs of Aronia melanocarp (also known as wild cherry berry and ageless berry) by enzymatic method. Anthocyanins are a kind of natural plant pigments, water-soluble, it gives our daily fruits, flowers, vegetables color, mostly in the form of glycosides, can be used for food coloring. The results showed that the extraction rate of anthocyanins was the highest with single enzyme Pectinex XXL (0.5%), and 2082.41 ± 85.69 mg/100 g was obtained, which proved the high efficiency of enzymatic extraction.

Nikoletta Solomakou et al.[31] combined ultrasonic-assisted extraction with enzymatic extraction to extract valuable by-products from pomegranate. Using pomegranate seeds as experimental materials, four single factors were set up: enzyme

type, enzyme concentration, enzymatic hydrolysis time and liquid-solid ratio to explore the optimal extraction process. It is concluded that the best process and efficiency of ultrasound-assisted enzyme method are higher than other methods, such as single ultrasound-assisted extraction and supercritical fluid extraction.

Kapoor Shailendra[32] proposed that tectorigenin could inhibit the growth of liver malignant tumors by enhancing cell apoptosis. When tumor cells were exposed to tectorigenin, nuclear fragmentation occurred, and in addition, the expression of apoptosis signaling pathway caspase 3 was also increased. This proves that tectorigenin has anti-tumor effect.

1.3 Research contents and methods

1.3.1 Research content

(1) Consult the literature on the extraction of active ingredients of Chinese herbal medicine by enzyme method, and screen out the relevant enzymes. Common enzymes can be found from the enzymes used in several literatures, while paying attention to some parameters of enzymes: enzyme activity unit, acidity and alkalinity, grade category, production date and manufacturer, etc.

(2) To understand the basic situation of the Chinese herbal medicine Iridis Tectori Rhizoma and tectorigenin. Iridis Tectori Rhizoma is the dried rhizome of Iris in the iris family, and tectorigenin is the bioactive component of Iridis Tectori Rhizoma.

(3) According to the mechanism of enzyme action and extraction elements, acid protease, acid cellulase, xylanase and pectinase (II) were added to carry out enzymolysis reaction, and the enzymolysis effects of various enzymes were investigated.

(4) One of the best factors can be obtained through single factor experiment and HPLC detection in each round. Through several rounds, the optimum process parameters of enzymatic extraction were obtained.

1.3.2 Research methods

(1) Literature method: As the name implies, look for relevant literature through the knowledge network or the library, find out the relevant common enzymes, and pay attention to the parameters.

(2) Pre-design method of this experiment (brief) :

Material treatment: Dry the Iridis Tectori Rhizoma without crushing.

Enzymatic reaction: Add appropriate amount of solvent, stir evenly, add acid protease, acid cellulase, xylanase and pectinase (II) respectively, set the temperature and pH, and carry out enzymatic reaction.

Product extraction: the mixture after enzymatic hydrolysis is centrifuged and the supernatant is collected. After concentration, the concentrated liquid was obtained.

Product detection: High performance liquid chromatography (HPLC) was used to determine the concentration of tectorigenin.

1.4 Expected results and significance

1.4.1 Expected Results

On the basis of several single factor experiments and blank control experiments, the optimal extraction process of the enzyme extraction of tectorigenin was obtained by high performance liquid chromatography (HPLC), including what kind of enzyme was used, what kind of enzymatic temperature, pH and enzymatic time. Compared with the traditional Chinese medicine extraction method, the extraction efficiency was greatly improved.

1.4.2 Meaning

(1) Theoretical significance

This topic is based on the cross study of "enzyme extraction" and "natural active substance tectorigenin in Chinese herbal medicine Iridis Tectori Rhizoma", which has certain theoretical research value and can further enrich the database of natural active substances extracted by enzyme.

(2) Practical significance

In response to the national call of "vigorously promoting the development of veterinary Chinese medicine industry and further developing traditional veterinary Chinese medicine", this paper uses enzymatic method to extract tectorigenin from Iridis Tectori Rhizoma and obtains the best extraction technology. This process can provide a theoretical basis and reference for the production of Chinese herbal medicine additives in animal feed manufacturing industry to simplify the production process, reduce the loss of medicinal materials and improve the production efficiency, and can be further applied to industrial production in the future.

Conclusions to chapter 1

1. The state controls the addition of antibiotic additives to animal feed, and advocates the use of Chinese herbs instead of antibiotics to be added to animal feed.
2. Chinese herbal medicines benefit from the active substances in them, but the extraction of active sub stances needs to break through the obstacles of cell walls. Enzymatic degradation of cell wall was studied.
3. Two kinds of active substances contained in the Chinese herbal medicine Iridis Tectori Rhizoma: tectoridin and tectorigenin have good efficacy, and can be used as additives in animal feed to enhance the resistance of the host.

CHAPTER 2

OBJECT, PURPOSE AND METHODS OF THE STUDY

2.1 Experimental materials

(1) Experimental medicinal materials

Authentic herbal medicine Iridis Tectori Rhizoma, from Shandong Jinan Jinzhuji Pharmaceutical Co., LTD. Chinese herbal medicine base.

(2) Experimental reagents

Table 2.1 - **Lists the reagents**

Reagent name	Specification	Manufacturer
1	*2*	*3*
Acid cellulase	200,000 U/g	Shandong Longcott enzyme Preparation Co., LTD
Acid protease	800,000 U/g	Shandong Longcott enzyme Preparation Co., LTD
Xylanase	100,000 U/g	Shandong Longcott enzyme Preparation Co., LTD
Pectinase (II)	30,000 U/g	Shandong Longcott enzyme Preparation Co., LTD
Ethanol (anhydrous ethanol)	Analytically pure	Sinopharm Group Chemical reagent Co., LTD
Carbinol	Analytically pure	Sinopharm Group Chemical Reagent Co., LTD
Phosphoric acid	Analytically pure	Sinopharm Group Chemical Reagent Co., LTD
Purified water	GB 17323	Wahaha Hongzhen Drinking water Co., LTD
Disodium hydrogen phosphate dodecahydrate	Analytically pure	Sinopharm Group Chemical Reagent Co., LTD

Continuation of Table 2.1

1	*2*	*3*
Disodium hydrogen phosphate dodecahydrate	Analytically pure	Sinopharm Group Chemical Reagent Co., LTD
Citric acid monohydrate	Analytically pure	Sinopharm Group Chemical Reagent Co., LTD
Mixed phosphate	Analytically pure	Shanghai Hongbei Reagent Co., LTD
Sodium tetraborate	Analytically pure	Shanghai Hongbei Reagent Co., LTD
Potassium hydrogen phthalate	Analytically pure	Shanghai Hongbei Reagent Co., LTD

2.2 Experimental Instruments

Table 2.2 - **Lists the instruments**

Instrument name	Manufacturer
Electronic analytical balance	Nanjing Bonita Scientific Instrument Co., LTD
Sartorius scales	Sartorius Scientific Instruments (Beijing) Co., LTD
Electric thermostatic Water Bath (DK-S26)	Shanghai Jinghong Experimental Equipment Co., LTD
Centrifuge	Shanghai Mycelium Separation Technology Co., LTD
Ultrasonic crusher	Huatai ultrasonic cleaning machine Co., LTD
pH meter	Bell Analytical Instruments (Dalian) Co., LTD
Liquid chromatograph	Anhui Wanyi Technology Co., LTD
Rotary evaporator (RE-52A)	Shanghai Yarong biochemical instrument factory
Circulating water Vacuum Pump (SHZ-III)	Shanghai Xiande Experimental Instrument Co., LTD
Pipette gun	Thermo Fisher Technologies

2.3 Experimental methods

2.3.1 Preparation of pH buffer

The preparation of pH buffer can provide pH conditions for enzymatic hydrolysis experiment. It was prepared with sodium hydrogen phosphate ($Na_2HPO_4 \cdot 12H_2O$) and citric acid ($C_6H_8O_7 \cdot H_2O$) monohydrate. The following are some parameters: $Na_2HPO_4 \cdot 12H_2O$ molecular weight = 358.22; The 0.2 mol/L solution is 71.64 g/L. $C_6H_8O_7 \cdot H_2O$ molecular weight = 210.14; The solution of 0.1 mol/L is 21.01 g/L. A buffer with a pH of 3.6 has a volume ratio of 6.44:13.56. The volume ratio of a buffer with a pH of 6.0 is 12.63:7.27.

As shown in experimental method 2.3.2 and Table 2-4, each group in the parallel experiment needs to be equipped with a pH buffer of 200 ml, and the required ratio of sodium hydrogen phosphate ($Na_2HPO_4 \cdot 12H_2O$) solution to citric acid monohydrate ($C_6H_8O_7 \cdot H_2O$) solution in different pH buffers is different.

In the buffer solution with a pH of 3.6, 64.4 ml of deionized water was measured with a measuring cylinder, and 4.61 g of disodium hydrogen phosphate dodecahydrate was weighed with a precision electronic balance.

The drug was mixed with deionized water and stirred with a glass rod until the drug was completely dissolved in the deionized water, and $Na_2HPO_4 \cdot 12H_2O$ solution was prepared. Measure 135.6 ml of deionized water, weigh 2.85 g of citric acid monohydrate, stir well, and prepare $C_6H_8O_7 \cdot H_2O$ solution. Mix the two solutions to create a pH 3.6 buffer.

See the following table for details:

Table 2.3 - **Lists the configurations of the two drugs**

pH	$Na_2HPO_4 \cdot 12H_2O$		$C_6H_8O_7 \cdot H_2O$	
	Mass/g	Volume/ml	Mass/g	Volume/ml
3.6	4.61	64.4	9.05	126.3
6.0	2.85	135.6	1.53	72.7

In the buffer solution with pH 6.0, 126.3 ml of deionized water was measured and 9.05 g of disodium hydrogen phosphate was weighed to form $Na_2HPO_4 \cdot 12H_2O$

solution. Measure 72.7 ml of deionized water, weigh 1.53 g of citric acid monohydrate, and prepare $C_6H_8O_7 \cdot H_2O$ solution. The mixture of the two solutions is a pH 6.0 buffer.

2.3.2 Preparation of extract sample

Four enzymes: acid protease, acid cellulase, xylanase and pectinase (II) were used to enzymolysis Chinese medicinal materials Iridis Tectori Rhizoma. Each group was added 10 g of Iridis Tectori Rhizoma (without grinding), and the enzyme was added according to 2% of Iridis Tectori Rhizoma mass (0.2 g), the optimal pH of the enzyme and the enzymatic hydrolysis temperature were searched through other channels, and a blank control group was added, that is, no enzyme was added. A pair of parallel experiments were performed for each enzyme and the control group, with a total of 10 groups. For specific Settings, see Table 2-4 and 2-5 below:

Table 2.4 - **Single enzyme hydrolysis setting table**

Enzyme name	pH	Temperature/℃	Solvent name	Volume/ml
Acid protease	3.6	50	Buffer fluid	200
Acid cellulase	3.6	50	Buffer fluid	200
Xylanase	6.0	50	Buffer fluid	200
Pectinase (II)	3.6	50	Buffer fluid	200
Blank	Blank	50	Deionized water	200

Table 2.5 - **Group ids**

Group number	Enzyme name
1	*2*
0^1	Blank
0^2	Blank
1^1	Acid protease

Continuation of Table 2.5

1	*2*
1^2	Acid protease
2^1	Acid cellulase
2^2	Acid cellulase
3^1	Xylanase
3^2	Xylanase
4^1	Pectinase (II)
4^2	Pectinase (II)

(1) According to the contents in Table 2.4 and Table 2.5, the corresponding proportion of medicinal materials, enzymes and solutions were prepared in 10 conical bottles, and the group number was marked. Each group was stirred with glass rods for 2 min. The 10 groups were placed in a thermostatic water bath with a set temperature of 50 ℃ and a water bath time of 4 h. After the water bath, place on the test bench and let cool. After cooling, the extraction liquid is collected by filtering the residue with a filter screen and stored in another new bottle. 10 groups of samples were marked with black marker on the pipette, i.e. 0^1, 0^2, 1^1, 1^2, 2^1, 2^2, 3^1, 3^2, 4^1, 4^2, and stored in refrigerator.

(2) The remaining 10 groups of extracts were combined in pairings according to parallel experiments to obtain 5 groups of extracts, namely 0^{1+2}, 1^{1+2}, 2^{1+2}, 3^{1+2}, 4^{1+2}. The 5 groups of extracts were placed in a rotary evaporator, concentrated 3 times at 60 ℃ for 1 h, and the volume before and after concentration were recorded. After concentration is complete, use a black marker on the pipette labeled $0^{1+2,}$, $1^{1+2,}$, $2^{1+2,}$, $3^{1+2,}$, $4^{1+2,}$, and store in the refrigerator.

(3) The Chinese medicine residue in 10 groups of original conical bottles was added into 250.ml deionized water and stirred with glass rod for 2.min. 5 groups of Chinese medicine residue, namely 0^1, 1^1, 2^1, 3^1 and 4^1, were re-labeled as a^1, b^1, c^1, d^1 and e^1, and were put into the ultrasonic crusher for 5 min. After completion, remove and use the filter to obtain the extract and store it in a new bottle. The extracted liquid

in these 5 new bottles was concentrated 5 times in a rotary evaporator at a set temperature of 60 ℃ for 1h concentration time, and the volume before and after concentration was recorded. After concentration is complete, labels $a^{1'}$, $b^{1'}$, $c^{1'}$, $d^{1'}$, $e^{1'}$ .Store in the refrigerator.

(4) Take the other 5 groups, namely 0^2, 1^2, 2^2, 3^2 and 4^2, re-label them as a^2, b^2, c^2, d^2 and e^2, and place them in a constant temperature water bath at a set temperature of 50 ℃ for 4 h. When finished, remove, let stand, filter the extract and transfer it to a new bottle. After that, the concentration was 5 times in the rotary evaporator, the temperature was 60 ℃, the time was 1h, and the volume before and after the concentration was recorded. After concentration is complete, store in the refrigerator with labels $a^{2'}$, $b^{2'}$, $c^{2'}$, $d^{2'}$, $e^{2'}$.

(5) After steps (1), (2), (3) and (4), a total of 25 groups of samples were obtained.

2.3.3 HPLC detection and standard curve drawing

(1) Detection principle

In this study, High Performance Liquid Chromatography (HPLC) was used to detect tectorigenin in the extract. High performance liquid chromatography, also known as high pressure liquid chromatography, as the name suggests, uses a high pressure infusion system in which the mobile phase is a liquid (where the liquid can choose a single solution or a solution rich in multiple substances). The liquid mobile phase is loaded into the chromatographic column containing the stationary phase, and the substances in the solution to be tested will be separated and then detected by the detector to obtain specific relevant information.

(2) Preparation of mobile phase by HPLC

pH meter calibration: The use of a sensitive pH meter will improve the efficiency and accuracy of the experiment. Here, mixed phosphate (pH 6.86, 25 ℃), sodium tetraborate (pH 9.18, 25 ℃) and potassium hydrogen phthalate (pH 4.00, 25 ℃) are configured into 250 ml solution as a pH standard solution to detect pH meters.

Mobile phase configuration: water (phosphoric acid to pH 3) - methanol (45：55) mixed solution is used to configure the mobile phase, provisionally configured with 1L. First use a measuring cylinder to measure 450 ml of water, and then use phosphoric acid to modulate pH 3, the volume may increase, and finally retain 450 ml. After measuring 550 ml methanol, it is mixed with water to form 1000 ml mobile phase. The mobile phase should be filtered by a vacuum filter, and after completion, it should be ultrasounded by an ultrasonic machine for 30 min. If 2 L mobile phase is configured later, 1 h ultrasound is performed.

(3) Standard curve drawing

Preparation of standard samples: electronic balance is used to accurately weigh certain quality of tectoridin and tectorigenin standard products as solutes. Then methanol was used as the solvent to dissolve the standard product respectively, and 500 ug/ml of the reserve liquid containing tectoridin and tectorigenin was prepared. Then a certain amount of the reserve liquid was taken and diluted with the mobile phase prepared in 2.3.3 (2) to form a standard sample solution containing tectoridin and tectorigenin at concentrations of 0.2, 0.5, 1, 2.5, 5, 7.5 and 10 ug/ml. There are 7 groups in total.

The sample solution of 7 groups of standard samples was tested by HPLC to make the standard curve. The setting conditions of HPLC were based on the method in the literature "Simultaneous Determination of tectoridin and tectorigenin in Erectin Extract by HPLC" [33] : The chromatography was performed on a Kromasil C18 column (250 mm×4.6 mm, 5 μm) with water (pH 3 adjusted by phosphoric acid) -methanol (45：55) as the mobile phase at the flow rate of 0.8 mL/min. The column temperature was 35 ℃ and the detection wavelength was 265 nm. The method is simple, rapid and accurate, and can be used to determine the contents of tectoridin and tectorigenin simultaneously.

2.3.4 Concentration of extraction solution

The use of Rotary Evaporator concentration operation, the principle of concentration is to reduce pressure, will reduce the conventional boiling point of the liquid to be tested, so that the liquid to be tested can be evaporated at a lower temperature. Operation steps:

Assembly: The distillation flask, heating pot, condensing tube, frame, rotary motor and other components are assembled. Note that the vacuum system is properly connected and does not leak.

Turn up: The liquid to be concentrated is added to the distilling flask and the top runner is rotated to adjust the optimum height.

Parameter: Set the temperature 60-65 ℃, the maximum does not exceed 7 ℃. The concentration time is set to about 1 h (Can be adjusted according to the specific situation).

Collection: After concentration is complete, turn off the vacuum system, slowly remove the distillation flask, pour into the measuring cylinder to measure the volume.

2.3.5 Ultrasonic crushing

Ultrasonic cell crusher was used for extraction. The principle is that ultrasonic waves have three major effects (cavitation, heat, and mechanical). Cavitation effect means that ultrasound will cause small bubbles in the liquid, and the small bubbles will burst immediately, quickly releasing energy, which can break the cells and release the contents. Thermal effect refers to the molecular vibration caused by ultrasonic transfer in solution, and the heat will be released due to friction between molecules. The mechanical effect is that the regularity of ultrasound fluctuations during transmission will create a pressure difference that can lyse cells.

5 groups in 2.3.2 (3) were selected, namely a^1, b^1, c^1, d^1 and e^1. The enzymatic hydrolysis solution was filtered out from each group, and 250 ml deionized water was added into the drug residue, which was placed into the ultrasonic crusher. The ultrasonic time was set at 5 min, the ultrasonic power was set at 360 W, and the ultrasonic temperature was set at 25 ℃, which was the natural temperature.

2.3.6 Detection of total samples

(1) Pretreatment of extract sample before detection

Centrifugation: The 25 sets of samples obtained in 2.3.2 were subjected to centrifuge operation prior to HPLC testing because precipitation stratification would occur after being stored in the refrigerator. 1.5ml was taken from 25 groups of samples and placed in a 2ml centrifuge tube. The centrifuge was set at a centrifuge temperature of 4 ℃ and a rotating speed of 6000 rpm for 3 min.

Dilution: After centrifugation, extract each group of supernatants. Use a pipette gun to measure 100ul of supernatant, and then add 900 ul of ultra-pure water, that is, dilute 10 times.

Extraction and filtration: After dilution, use a syringe to extract 1ml diluent, through the filter membrane, injected into the liquid phase detection vial, and marked with black marker.

(2) Pretreatment of standard samples

Because the standard sample is relatively pure, it only needs to go through the dilution and extraction filtration operations in (1).

(3) Total sample testing

A total of 32 sets of liquid phase test vials are put into the liquid phase feed sample according to the preparation in 2.2.3. Pay attention to the self-contained label on the feed sample for testing and obtain the peak map.

Conclusions to chapter 2

1. In this experiment, the enzymolysis effect of four enzymes was investigated as the first treatment, and ultrasound and water extraction as the second treatment.
2. After making samples, label them separately. Different groups of follow-up treatment are different, some through the ultrasonic crusher, some through the constant temperature water bath, some through the rotary evaporation meter, and finally unified through the centrifuge, and then through the liquid chromatograph to detect the peak area.

CHAPTER 3
EXPERIMENTAL PART

3.1 Enzymatic hydrolysis and sampling pictures

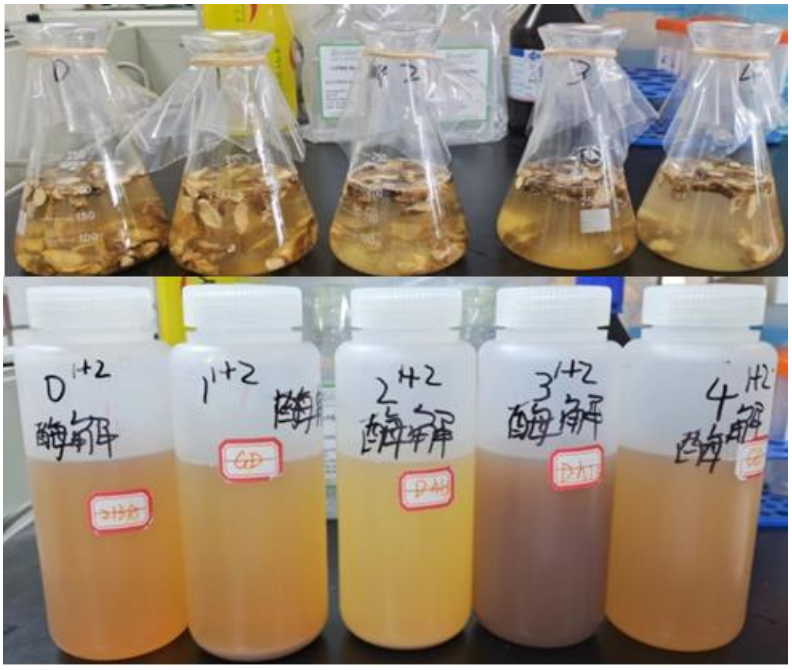


Figure 3.1 - **Appearance of the enzymolysis sample**

Note: Top from left to right are blank control group 0^1, acid protease group 1^1, acid cellulase group 2^1, xylanase group 3^1 and pectinase (II) group 4^1. The bottom row shows the filtrated extract of each group.

3.2 Condensing data with rotary evaporator

Table 3.1 - **Records of concentration volume of enzymolysis solution**

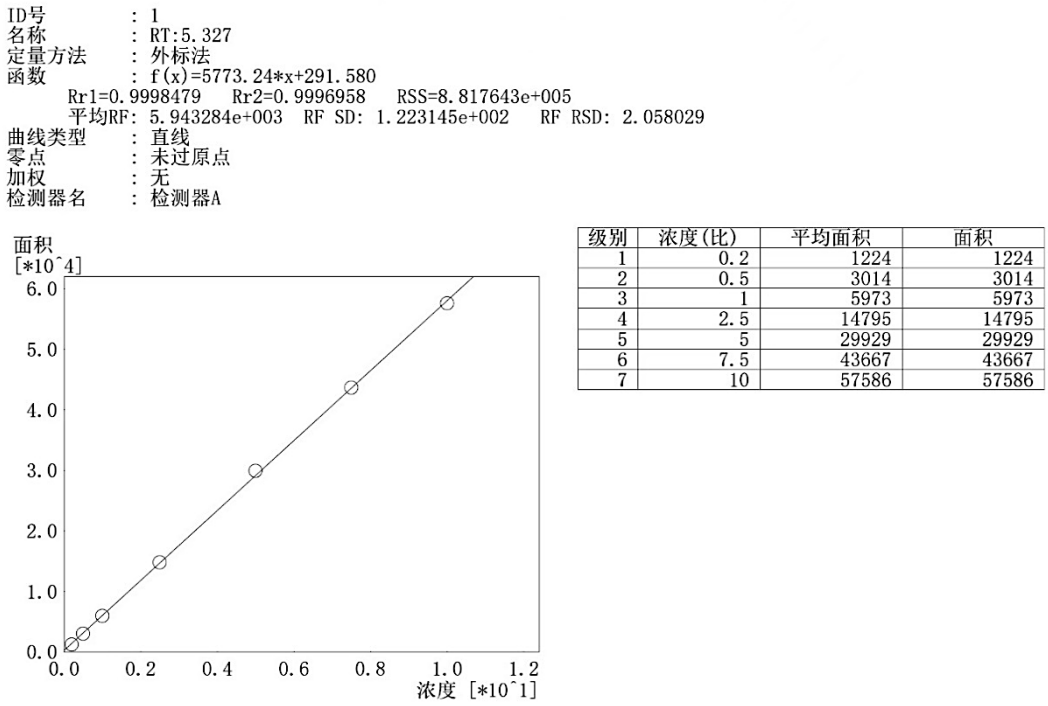
Group number	0^{1+2}	1^{1+2}	2^{1+2}	3^{1+2}	4^{1+2}
Before concentration, ml	325	320	305	305	310
Group number	$0^{1+2,}$	$1^{1+2,}$	$2^{1+2,}$	$3^{1+2,}$	$4^{1+2,}$
After concentration, ml	105	108	100	108	95

Table 3.2 - **Deionized water concentration volume records**

Group number	a^1	b^1	c^1	d^1	e^1	a^2	b^2	c^2	d^2	e^2
Before concentration, ml	240	245	240	235	235	237	235	237	235	237
Group number	$a^{1,}$	$b^{1,}$	$c^{1,}$	$d^{1,}$	$e^{1,}$	$a^{2,}$	$b^{2,}$	$c^{2,}$	$d^{2,}$	$e^{2,}$
After concentration, ml	60	55	55	57	60	53	60	60	61	55

3.3 Standard Curve

3.3.1 Tectoridin standard curve



级别	浓度(比)	平均面积	面积
1	0.2	1224	1224
2	0.5	3014	3014
3	1	5973	5973
4	2.5	14795	14795
5	5	29929	29929
6	7.5	43667	43667
7	10	57586	57586

Figure 3.2 - **Standard curve of tectoridin**

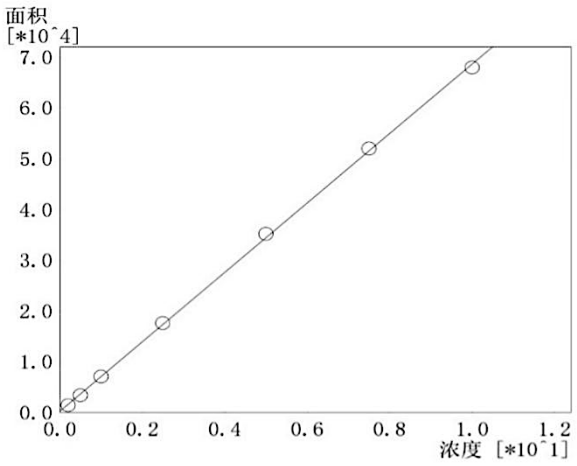
3.3.2 Standard curve of tectorigenin

2.3.3 (3) was adopted to perform linear regression with peak area as the ordinate and injection concentration (0.2-10 ug/ml) as the abscissa. The results were shown in Figure 3.2 and Figure 3.3. The injection concentration had a good linear relationship with peak area within the corresponding concentration range, and the regression equations were as follows:

$F(x)_{tectoridin} = 5773.24 + 291.580 \times (r = 0.999)$ · · · · · · · · · · · · · · · · · formula 1

$F(x)_{tectorigenin} = 6831.64 + 303.052 \times (r = 0.999)$ · · · · · · · · · · · · · · formula 2

ID号 : 2
名称 : RT:13.570
定量方法 : 外标法
函数 : f(x)=6831.64*x+303.052
Rr1=0.9998374 Rr2=0.9996749 RSS=1.319788e+006
平均RF: 6.958977e+003 RF SD: 1.180331e+002 RF RSD: 1.696127
曲线类型 : 直线
零点 : 未过原点
加权 : 无
检测器名 : 检测器A



级别	浓度(比)	平均面积	面积
1	0.2	1407	1407
2	0.5	3404	3404
3	1	7086	7086
4	2.5	17583	17583
5	5	35155	35155
6	7.5	51950	51950
7	10	67941	67941

Figure 3.3 - **Standard curve of tectorigenin**

3.4 Extraction results of single enzyme hydrolysate

3.4.1 Analysis of single enzyme extraction amount

According to Figure 3.4, the extraction amount of tectorigenin in the acid cellulase group was the highest, reaching 89.8 ug/g. The reason may be that the main component of the cell wall of most plants is cellulose, so cracking the cell wall cellulase is essential, and many similar enzymatic methods to extract the active ingredients in Chinese herbs have at least used cellulase. The extraction rate of the blank control group was 60.5ug/g, and that of the xylanase group was 60.0 ug/g, which was not much different from that of the blank control group. The extraction rates of acid protease group and pectinase (II) group were 47.7 ug/g and 50.0 ug/g, respectively, which were lower than those of blank control group. The reason may be that in the actual operation process, the leakage of the enzymolysis sample will lead to the loss of the sample, and the chromatographic peak will decrease compared with the normal determination result,

which will lead to the reduction of the enzymolysis extraction amount.

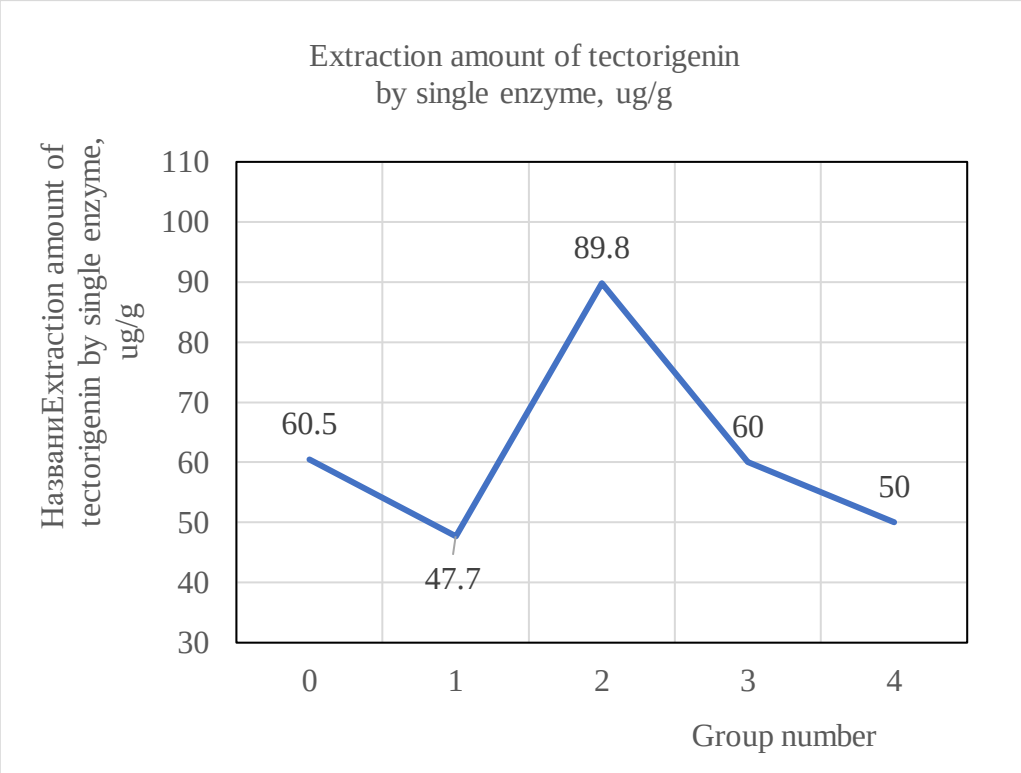


Figure 3.4 - **Comparison of extraction amount of single enzyme tectorigenin**

Note: From left to right, there were blank control group, acid protease group, acid cellulase group, xylanase group and pectinase (II) group. Each group was treated twice. The first treatment was to filter out the enzymolysis solution by enzymolysis at a water bath temperature of 50 ℃ and a water bath time of 4 h. The second treatment is to add 250 ml deionized water to each group of pharmaceutical residues, and then carry out ultrasonic-assisted extraction under the conditions of 360 W ultrasonic power, 25 ℃ ultrasonic temperature, and 5min ultrasonic time. Finally, the content of irisflavin was determined by HPLC peak area, and the extraction amount (ug/g) was obtained.

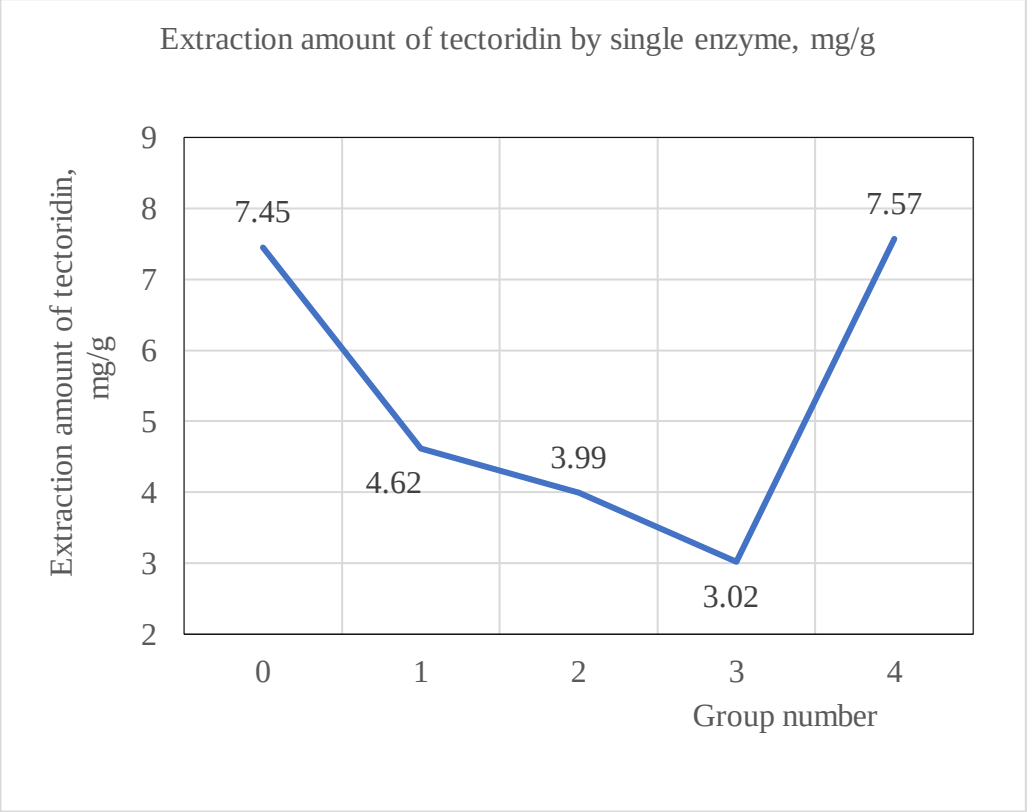


Figure 3.5 - **Comparison of extraction amount of single enzyme tectoridin**

Note: From left to right, there were blank control group, acid protease group, acid cellulase group, xylanase group and pectinase (Ⅱ) group. Each group was treated twice. The first treatment was to filter out the enzymolysis solution by enzymolysis at a water bath temperature of 50℃ and a water bath time of 4h. The second treatment is to add 250ml deionized water to each group of pharmaceutical residues, and then carry out ultrasonic-assisted extraction under the conditions of 360W ultrasonic power, 25℃ ultrasonic temperature, and 5min ultrasonic time. Finally, the extraction amount (mg/g) was obtained by HPLC peak area.

According to Figure 3.5, the extraction amount of tectoridin in the blank control group was 7.45 mg/g, and that of pectinase (Ⅱ) group was as high as 7.57 mg/g, indicating that pectinase (Ⅱ) had a more significant effect on cell wall lysis, resulting in a large amount of tectoridin being released from cells. Relative to the acid protease group, acid cellulase group and xylanase group, the extraction amount of tectoridin was 4.62 mg/g, 3.99 mg/g and 3.02 mg/g, respectively, which was significantly different

from that of the blank control group and pectinase (Ⅱ) group, indicating that these three enzymes had poor effect in extracting tectoridin.

3.4.2 Comparison of extraction amount of tectoridin by ultrasound and water extraction

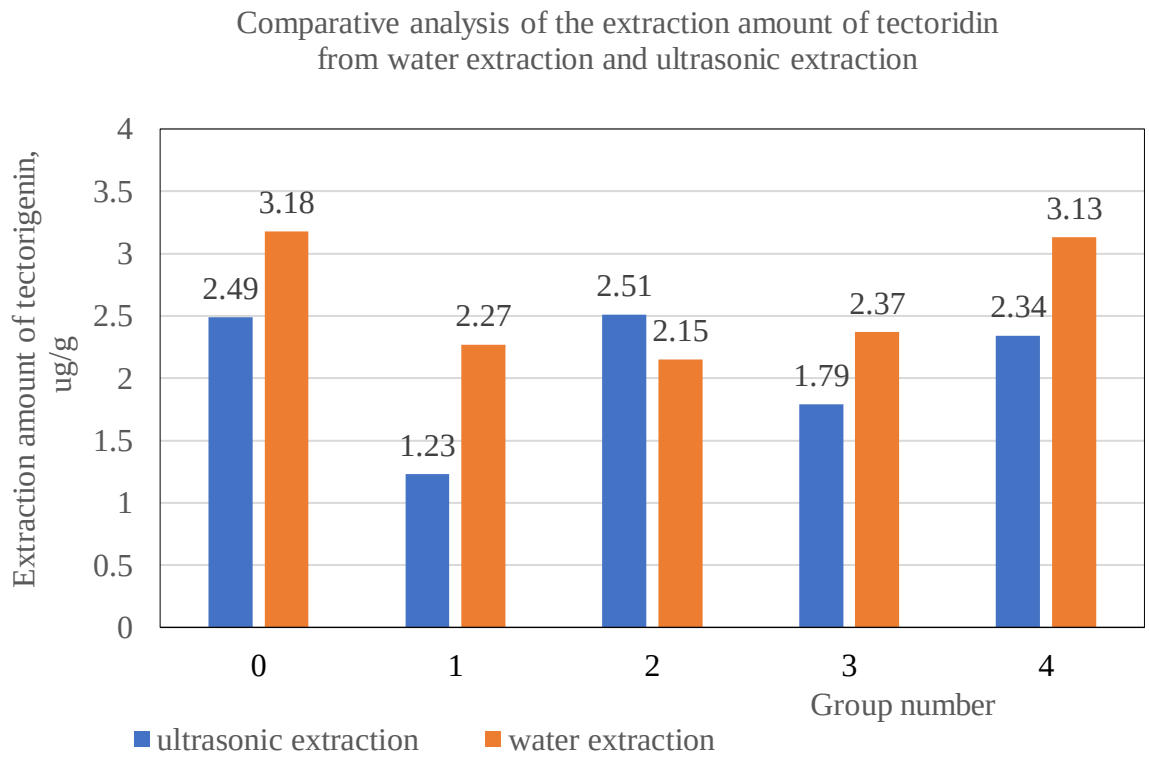


Figure 3.6 **- Comparison of the extraction amount of tectoridin in the two extraction methods**

Note: Both the ultrasonic group and the water extraction group were subjected to secondary treatment, that is, enzymatic hydrolysis reaction was performed first, and then enzymatic hydrolysis solution was filtered out (the first treatment), and the remaining residue was added to 250 ml of deionized water for ultrasonic or water extraction treatment. The setting conditions of the ultrasonic group were 360W ultrasonic power, 25 ℃ ultrasonic temperature and 5min ultrasonic time. The setting conditions of the water extraction group are water bath temperature 50 ℃ and water bath time 4h. From left to right, the two groups were blank control group, acid protease

group, acid cellulase group, xylanase group and pectinase (II) group. The ultrasound group labels are $a^{1'}$, $b^{1'}$, $c^{1'}$, $d^{1'}$, $e^{1'}$; The water extraction group is labeled $a^{2'}$, $b^{2'}$, $c^{2'}$, $d^{2'}$, $e^{2'}$.

According to FIG. 3-6, except for acid cellulase ultrasonic group b^1, the extraction amount of tectoridin was slightly higher than that of water extraction group b^2, the extraction amount of tectoridin in the other blank control group, acid protease group, xylanase group and pectinase (II) group was higher than that of ultrasonic extraction, but the difference was not significant. The extraction amount of tectoridin extracted by ultrasonography was relatively close to that by water extraction at 4 h after only 5 min. It can be seen that the high efficiency of ultrasonic extraction, if the ultrasonic action time is prolonged, the auxiliary enzymatic extraction will get a higher extraction amount of tectoridin.

3.4.3 Comparison of the extraction amount of tectorigenin by ultrasound and water extraction

According to Figure 3.7, the water extraction rate of the blank control group, the acid protease group, the xylanase group and the pectinase (II) group were all higher than the ultrasonic extraction rate, especially the blank control group and the pectinase (II) group, which may be due to the time difference: the ultrasonic group only set the ultrasonic time of 5min, while the water bath time of the water extraction group was 4 h. The effect of water extraction after 4 h is more significant than that after 5min. The extraction efficiency in the acid cellulase group was much higher than that in the water extraction group, and the improvement rate was increased by 91.8%, and there was no difference between the acid protease group and the xylanase group. The reason for the increase in extraction rate in the ultrasonic group was that under the enzymatic hydrolysis of acid cellulase, a lot of tectorigenin had been produced in the first treatment, but it remained in the cells. It was ultrasound that destroyed the complete structure of the cells, resulting in a large amount of tectorigenin release

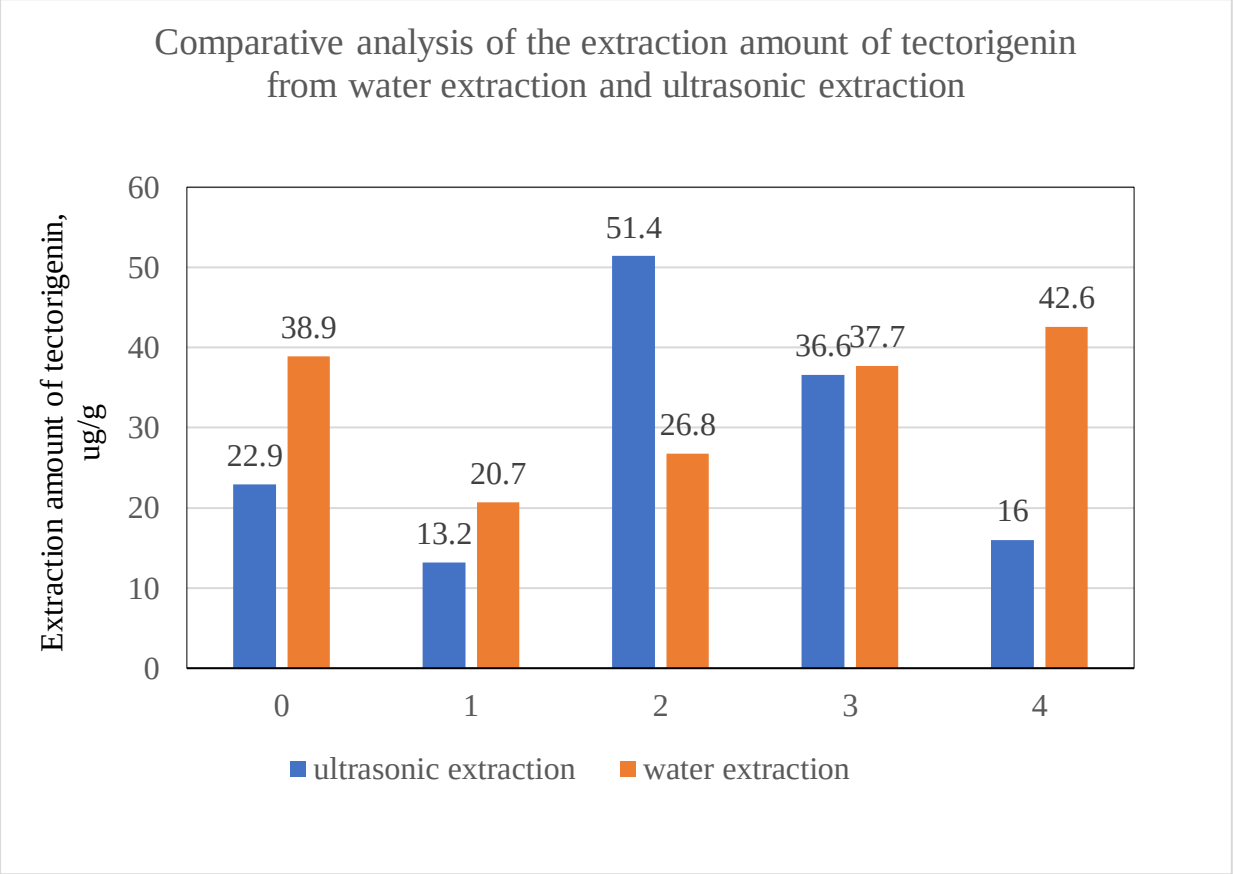


Figure 3.7 - **Comparison of the extraction amount of tectorigenin by two extraction methods**

Note: Both the ultrasonic group and the water extraction group were subjected to secondary treatment, that is, enzymatic hydrolysis reaction was performed first, and then enzymatic hydrolysis solution was filtered out (the first treatment), and the remaining residue was added to 250 ml of deionized water for ultrasonic or water extraction treatment. The setting conditions of the ultrasonic group were 360 W ultrasonic power, 25 ℃ ultrasonic temperature and 5min ultrasonic time. The setting conditions of the water extraction group are water bath temperature 50 ℃ and water bath time 4h. From left to right, the two groups were blank control group, acid protease group, acid cellulase group, xylanase group and pectinase (II) group. The ultrasound group labels are $a^{1,}$, $b^{1,}$, $c^{1,}$, $d^{1,}$, $e^{1,}$; The water extraction group is labeled $a^{2,}$, $b^{2,}$, $c^{2,}$, $d^{2,}$, $e^{2,}$.

Conclusions to chapter 3

1. By testing the standard substances, we obtained the standard equations of tectoridin and tectorigenin:

   $F(x)_{tectoridin}$=5773.24 + 291.580 x (r= 0.999) · · · · · · · · · · · · · · · · · formula 1

   $F(x)_{tectorigenin}$ = 6831.64 + 303.052 x (r = 0.999) · · · · · · · · · · · · · · · formula 2

2. By analyzing the enzymolysis samples, we found that the extraction amount of tec torigenin from acid cellulase was higher. Ultrasonic treatment can assist enzymatic method to increase the extraction amount of tectorigenin.
3. The enzymolysis effect of different enzymes is different.

CONCLUSIONS

It can be seen from the above experiments that compared with the direct water extraction method, the enzymatic treatment of Chinese herbal medicine can promote the extraction of active components to a certain extent, and the addition of ultrasonic-assisted means can greatly improve the transformation of small and medium-sized active components of Chinese herbal medicine. Different enzymes differ greatly in the dissolution efficiency of tectoridin and the conversion efficiency of tectorigenin. Among them, although pectinase has a higher dissolution rate of tectoridin, the dissolution rate of its active component, tectorigenin, is lower, while the dissolution rate of tectorigenin in acidic cellulase is significantly increased, thus confirming that appropriate enzymes can be used for enzymatic hydrolysis. It can effectively improve the extraction efficiency of the effective ingredients of Chinese herbal medicine and improve the actual efficacy of the preparation.

It can be seen from the above that the second ultrasonic treatment time is 5min, which is relatively short. Therefore, a set of single factor experiments can be conducted to extend the ultrasonic time to investigate the influence of different ultrasonic time on the extraction rates of radiosine and irisflavin. Can be set: 10min, 15min, 20min, 25min, 30min five groups.

Single enzyme treatment was used in this experiment. Different enzyme combinations can be used in future experiments. Considering the synergistic effect between various enzymes, different additive amounts of complex enzymes and the proportion of each enzyme of different complex enzymes can be set to investigate the effect of complex enzymatic hydrolysis.

As for the extraction temperature, it can be further optimized. In this experiment, 50 ℃ is uniformly set and all the enzymes used are proteins, and the temperature will affect the activity of the enzymes and thus the extraction effect of enzymatic hydrolysis.

Finally, the Iridis Tectori Rhizoma used in this experiment has not been crushed. In the future, the Iridis Tectori Rhizoma can be crushed into powder for enzymatic hydrolysis experiment, which can be compared with the results of this experiment.

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