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Research Progress on Extraction Technology of Gynostemma Pentaphyllum Saponins

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Abstract

College of Chemistry and Chemical Engineer Gynostemma saponin as an active component of gynostemma has medicinal value, it has certain curative effect on hypertension, obesity, hyperglycemia, hyperlipidemia and antioxidant, it is very valuable for research and development. This paper summarizes the extraction process and principle of noosin in recent years, and analyzes and compares the advantages and disadvantages of various extraction processes. Finally, the extraction process of gynostemma is discussed to provide reference for the comprehensive utilization and development of gynostemma.ing, Zhoukou Normal University, Zhoukou Henan, 466001, China.

Keywords

Gynostemma pentaphylla, saponin, extract, technology.

1. Introduction

Gypenoch is a common plant, mainly distributed in the subtropical and North subtropical regions, and in Asian countries such as China, Japan, Korea, Korea, Thailand, Vietnam and Malaysia [1]. There are 16 species and 2 varieties of Gypenoenoides found worldwide, and in China, this number reaches 15 species and 2 varieties[2].Gypenoylum heterophyllum can be used as medicine in whole grass. It is rich in polysaccharides, flavonoids, saponins and amino acids, which can improve the growth and disease resistance of plants. The total saponins of Gypenoenoside contain 300 different compounds [3], which have the effects of anti-atherosclerosis [4], lowering blood lipids, protecting liver, improving fatty liver [5], lowering blood sugar, delaying aging, anti-cancer [6], improving brain vitality [7], protecting optic network nerve [8], and reducing weight [9]. Therefore, Gypenosum is often developed as tea, gypenosum total glycosides, gypenosum beverage, gypenosum oral liquid and other products, with good medical value and market value. The extraction methods of gypenosides are usually water extraction, enzyme, ultrasonic and microwave. Gypenoside is a kind of substance widely used in the research and development of health food and new drugs, which has important research and utilization value. The extraction technology of gypenosides has been concerned for a long time, and new technologies have been developed constantly. In this paper, the extraction technology of gypenosides in recent years was summarized, and various extraction technologies were analyzed and compared in order to provide reference for the comprehensive development and utilization of gypenosides.

2. Solvent extraction process

The method of extracting gypenosides with organic solvent or its mixed solvent is simple and efficient, but it requires a large amount of solvent, which brings some pollution to the environment. In addition, it is necessary to pay attention to the selection and collocation of solvents, as well as the subsequent decolorization, purification and other processes.

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2.1. Water extraction process

The solubility of gypenosides in water is high, and the use of water as solvent is beneficial to the extraction of saponins. Because gypenoside is a glycoside compound, water extraction also needs to add some additives such as sugar water, acid water and alkali water to promote the hydrolysis and stability of gypenogenin. Water extraction also needs to control different extraction conditions, such as extraction time, temperature, pH value and other factors, in order to achieve the best extraction effect. Gypenoside soap has certain thermal stability, but it is easy to decompose under high temperature and high pressure, so it is necessary to control the extraction conditions to ensure the stability and purity of saponins.

Lin Shuo [10] used water extraction to extract gypenosides, and conducted single factor experiments on the main influencing factors such as solid-liquid ratio, extraction time and extraction temperature to analyze the influence of these factors on the extraction rate of gypenosides. On the basis of the above single factor test results, orthogonal test was used to optimize the extraction process of gypenosides. The results showed that the main factors affecting the extraction efficiency of gypenosides were extraction temperature, solid-liquid ratio followed by time. The optimum process was set as solid-liquid ratio 1:35, temperature 85 ℃, extraction 90 min, the average saponin yield 7.7943%.

Water extraction process is low in cost and suitable for industrial large-scale production, but the water extraction liquid often contains inorganic salts, sugars, proteins and other water-soluble impurities with large polarity, in addition, water as an extraction solvent is not easy to penetrate into the interior of plant cells, the effective components are difficult to dissolve, and the extraction rate is low.

2.2. Organic solvent extraction method

The organic solvent extraction method uses the affinity and solubility between the organic solvent and the compounds to be extracted to achieve the separation and enrichment of substances. The extraction efficiency and purity can be adjusted by changing the pH value of the solution, the type and concentration of the extractant, and the extraction time.

Liu Xinyan [11] studied the optimal extraction process of gypenosides by using 75% ethanol hot reflux method, single factor experiment and Box-Behken experiment. The optimized extraction process was as follows: solid-liquid ratio 1:50 g/mL, temperature 80 ℃, extraction time 1 h, recovery of 42.197 mg/100g.

Zhong Nana et al. [12] used ethanol as the solvent to extract gypenosides, and optimized the extraction process by single factor experiment and orthogonal test. The optimal extraction process was 70% ethanol, solid-liquid ratio 1:8, reflux heating for 45 min three times. Under these conditions, the extraction yield of gypenoside was 10.25%.

Cao Hui et al. [13] used ethanol as the solvent to extract gypenosides by percolation. The extraction technology of gypenoside was optimized by response surface method. The optimum extraction technology of gypenosides was obtained based on the extraction rate of total saponins, ethanol concentration, impregnation time and percolation flow rate. The experimental data were statistically processed by Design-Expert8.05 software. The optimal extraction process was 75% ethanol concentration, impregnation time 60 h, percolation flow rate 3.0 ml/min, and the yield of gypenoside was 6.59%.

In order to improve the extraction efficiency of gypenosides and total flavonoids, Yang Changhua et al. [14] evaluated the content of gypenosides and total flavonoids. The optimal extraction conditions for gypenosides and total flavonoids were investigated by single factor experiment. Methanol was selected as the extraction solvent, and the extraction time was 70 min, the solid-liquid ratio was 12:1, and the particle size was 120 mesh. The optimal extraction

process was 73% methanol, the extraction time was 40 min, the solid-liquid ratio was 1:15, and the average extraction rate of saponins was 13.12%.

However, the organic solvent extraction method also has shortcomings, such as poor wall breaking effect, long time, high energy consumption, high solvent cost, low recovery rate, and more losses, and it is necessary to assist other wall breaking technologies to overcome this weakness.

2.3. Water extraction and alcohol precipitation method

The water extraction and alcohol precipitation method takes advantage of the different solubility of saponins in water and alcohol phases. After adding water, the saponins in gypenosides will partially dissolve in water to form a water phase, and most of the gypenosides will dissolve in alcohol solvents such as ethanol to form an alcohol phase. Then, the saponins and other components are extracted by alcohol phase. Then the alcohol phase is treated with water, so that the saponins and other components are transferred to the water phase, and the alcohol phase is removed with organic solvents such as ethanol, and the precipitation of saponins is obtained.

Li Quanliang [15] first extracted gypenosides with boiling water and evaporated the extracted liquid to obtain 2.07g crude product, and then recrystallized the crude product with 95% ethanol to obtain 1.75g pure gypenosides, that is, the yield of gypenosides extracted by water extraction and alcohol precipitation was 1.75%.

The method of water extraction and alcohol precipitation can realize the efficient extraction of the active components in gynostaphyllum heterophyllum, which has the advantages of simple operation, low cost and friendly to the environment. However, the solvent selection of water extraction and alcohol precipitation method is limited, and only water and alcohol and other pro-polar solvents can be used for extraction, and non-polar organic solvents can not be used for extraction. At the same time, compared with other extraction methods, the solvent consumption of water extraction and alcohol precipitation method is larger.

3. Single assisted extraction process

3.1. Ultrasonic method

Ultrasonic extraction technology is a kind of technology using ultrasonic wave as energy source, using the high energy and mechanical effect of sound wave to separate, extract, chemical reaction and accelerate reaction. In the process of ultrasonic extraction of gypenosides, ultrasonic can produce high frequency compression wave, sparse wave and ultrasonic cavitation, so as to separate the saponins in gypenosides from other components.

In addition, ultrasound can also promote the diffusion and dissolution of reactants, accelerate the reaction rate and increase the mixing uniformity of the reaction system. Therefore, ultrasonic extraction technology is an efficient, fast and selective extraction method, especially suitable for the extraction and separation of active components in medicinal plants.

Du Hong et al. [16] used Box-Behnken to optimize the ultrasonic method, taking extraction time, ultrasonic power and the ratio of material to liquid volume as dependent variables, and the mass fraction of gypenoside in the extraction liquid as the response quantity, to study the optimal extraction process of gypenoside by ultrasonic method. It can be concluded that the optimum extraction conditions of gypenoside by ultrasonic method are as follows: under the conditions of power of 360 W, solid-liquid ratio of 1:60 g/mL, extraction for 15 min, the mass fraction of gypenoside is 24.7 mg/g.

Wang Lu et al. [17] used single factor method and Box-Behnken to discuss the optimal extraction process of gypenosides extracted by ultrasonic method. The optimal extraction

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process was as follows: ultrasonic power 300 W, extraction time 50 min, extraction temperature 60 ℃, ethanol volume fraction 60%, and the yield of gypenoside was 5.98%.

Lin Shuo et al. [18]using water as the solvent, placed the beakers containing the mixed liquid of capstock and water to be extracted into two ultrasonic extractors with a frequency of 40 kHz and 28 kHz respectively, and set the power at 500 W to conduct a single factor test. The results showed that 40 kHz ultrasound had no significant effect on the extraction efficiency of gypenosides, while 28 kHz ultrasound had a significant effect on the extraction efficiency of gypenosides. Under the condition of 28 kHz ultrasonic wave, the ratio of solid to liquid was 1:30, the extraction temperature was 70 ℃, and the extraction rate of gypenoside was 7.5934%.

The procedure of ultrasonic method is simple, the operation is simple, the equipment is not high, the extraction rate of saponin is increased, the extraction time is shortened. However, the extraction process is noisy, the extract is easy to deteriorate, and it is not suitable for large-scale industrial production.

3.2. Microwave extraction

The principle is to use the electromagnetic wave of microwave radiation to cause the ions and molecules in the material molecules to be affected by the internal friction in the high-frequency vibration state, so as to accelerate the temperature rise of the material and the chemical reaction rate. Under the aid of microwave, the saponins in Gypenoenoides were destroyed and dissolved quickly, and entered the extract, and the extraction effect was significantly better than that of traditional hot reflux extraction and ultrasonic extraction. In addition, the microwave method can also save time and energy, improve the purity and yield of the compound.

Zhang Yusong [19] used single factor test to extract gypenosides, and discussed the influence of various influencing factors on the extraction rate of saponins and the optimal extraction process. The results showed that microwave time and microwave intensity had great influence on saponins, and the optimal extraction process was as follows: solid-liquid ratio 1:25, solvent pH value 8, extraction temperature 70 ℃, extraction for 2 h, microwave treatment intensity medium, and microwave treatment time 3 min.

Guo Huili and Deng Zeyuan [20] used microwave dry method to extract gypenosides, and determined the optimal extraction power, microwave irradiation time and microwave-light wave combination method through orthogonal test, and compared it with the traditional ethanol reflux method. The optimum extraction process was determined as 800 W power, 100% microwave irradiation and 120 s irradiation time. The extraction rate of saponin was 8.37% under these conditions. Compared with the traditional ethanol reflux method, the time is shortened by about 9 h, and the saponin yield is increased by 15.8%.

3.3. Enzyme extraction method

Enzymatic extraction refers to the chemical separation technology using biological enzymes to selectively decompose and transform substances, which can be used to separate, purify and extract biological macromolecules, such as saponins in gypenosides.

The saponin in gypenoenoside is a cucurbituricoside compound composed of the glycoside group and the saponin core. The glycoside group contains more corrosion acid, while the saponin core contains a variety of glucosidase. Therefore, enzymatic extraction can be used to split the chemical bond between the saponin core and the glycoside group by the glucosidase to separate and purify the saponin.

Yi Changwen [21] conducted orthogonal experiments on the basis of single factor tests, taking extraction temperature, ethanol concentration, solid-liquid ratio and extraction time as influencing factors, to explore the influence of each influencing factor on the yield of gypenosides and the optimal extraction process of gypenosides. It was found that the factors

affecting the extraction rate of gypenosides were ethanol concentration > extraction temperature > solid-liquid ratio > extraction time when 0.5% cellulase was used for enzymatic hydrolysis at 55 ℃ for 1 h. The optimal process was that 0.5% cellulase was used for enzymatic hydrolysis of gypenosides at 55 ℃ for 1 h. Then at 70 ℃, ethanol concentration of 80%, solid-liquid ratio of 1:30, extraction for 25 min, the average extraction yield of gypenosides was 3.051%.

Lin Shuo et al. [22] used pectinase to extract gypenosides, studied the effects of enzyme dosage, pH value, enzymolysis temperature and enzymolysis time on the extraction rate of gypenosides, and optimized the extraction process with orthogonal method. Under the optimized process conditions, gypenosides were extracted by water bath heating and enzymolysis. Finally, the optimal process of using pectinase to extract gypenosides was obtained by adding 0.35% pectinase, pH value was 4.0, enzymolysis temperature was 50 ℃ on the basis of enzymolysis 90 min, and finally high temperature enzymolysis 16 min. Based on the above conditions, the extraction yield of gypenoside reached 7.9201%, which was 9.4148% higher than that of conventional enzymatic extraction.

The enzyme extraction method has the advantages of simple operation, little influence on other components such as protein and high extraction efficiency, but it also has some disadvantages such as high enzyme cost, limited enzyme operation conditions and unsuitable waste disposal.

4. Compound assisted extraction method

4.1. Ultrasonic-microwave extraction

Ultrasonic and microwave combined extraction technology is a chemical separation technology that combines ultrasonic and microwave. This method can make full use of the mechanical effect of ultrasonic wave and the heating effect of microwave, and realize the efficient extraction of saponins from Gypenoside.

In the ultrasonic-microwave extraction technology, the samples of gynostemma were first added to the suitable extraction solvent, and then the samples were placed in the ultrasonic-microwave extraction instrument. Ultrasonic wave can produce high energy local eddy current and polarization effect, make the molecules inside the sample vibrate, and accelerate the diffusion and dissolution of saponin components. Microwaves can gently heat the sample and promote the healing and diffusion of the saponins in the gypenosides. Through the combination of ultrasonic and microwave, the extraction time can be greatly shortened, the extraction efficiency can be improved, the extraction temperature can be reduced, and the destruction of the active ingredients in the sample can be reduced. At the same time, it can also reduce the pollution and legacy of other substances brought about by the upflow and oscillation effects, and extract the purity and quality of substances.

Cheng Yiqun et al. [23] conducted a single factor test to investigate the sequence of influence factors on saponin yield under the conditions of ultrasound for 50 W, fixed ethanol concentration of 70%, and solid-liquid ratio of 1:20 g/mL. Based on the results of single factor test, response surface test was used to optimize the ultrasonic-microwave extraction method, and the effect of ultrasonic-microwave on the extraction rate of saponin was analyzed. Under the conditions of microwave power 613 W, microwave time 145 s and extraction temperature 62 ℃ for 12.8 min, the extraction yield of gypenosides was (24.08±0.37) mg/g. At the same time, it was found that ultrasonic and microwave had synergistic effect on the extraction rate of gypenosides, and the synergistic effect of ultrasonic and microwave played an important role in the extraction process.

Ultrasonic microwave combined extraction of gypenosides has the advantages of high extraction efficiency, short extraction time, energy saving, environmental protection and

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economy, but it also has some disadvantages such as high equipment cost, high technical requirements and may affect the stability of components.

4.2. Ultrasonic assisted two aqueous phase

Ultrasonic-assisted two-phase aqueous method is a common low temperature extraction method, which can achieve high efficiency extraction of gypenosides. The principle is based on the two-phase aqueous system, using the different interaction of two insoluble solutions to transfer gypenosides from the aqueous phase to the organic phase, so as to achieve the purpose of extracting the target substance. Ultrasonic was added to the extraction process by ultrasonic-assisted two-phase aqueous method, and the mechanical vibration of ultrasonic was used to accelerate the diffusion of solvent and the migration of substances, so as to improve the extraction rate.

Zhou Biao et al. [24] first prepared six different aqueous two-phase systems, and selected the solvent system with the highest yield based on the yield of gypenoside, that is, the extraction solvent of 30% gypenoside was 30% ethanol-20% ammonium sulfate. Secondly, microwave assisted 30% ethanol-20% ammonium sulfate two-phase method, single factor method and Box-Behnken method were used to study the optimal extraction technology of gypenosides. The optimum technology was as follows: solid-liquid ratio 1:28 g/mL, ultrasonic time 50 min, extraction temperature 50 ℃, the yield of gypenoside was 7.91%.

The ultrasonic-assisted two-phase aqueous extraction of gypenosides has the advantages of high extraction efficiency, wide application range and simple operation, but it also has some disadvantages such as complicated process, low selectivity and influence of aqueous environment on samples.

4.3. Microwave-assisted enzymatic method

Microwave assisted enzymatic method is a chemical reaction method that combines the electromagnetic energy of microwave field with the enzymatic reaction, which can be used to extract the active ingredients in medicinal plants, such as saponins in gypenosides.

In the microwave-assisted enzymatic process, gypenoylum powder or its extract is first added to a buffer containing the desired enzyme, and then the reaction system is heated in a microwave oven. The energy of the microwave field can increase the rate and energy of the molecules in the reaction system, thus accelerating the catalytic action of enzymes and promoting the formation of products. At the same time, microwave can also make solvent molecules excited, improve the speed and yield of chemical reaction.

Zhang Di et al. [25] selected pectinase, cellulase and hemicellulase to allocate complex enzymes according to the distribution location of the special motor saponins in the cell wall of gypenoside, in order to give full play to the advantages of each enzyme, and assisted by microwave to destroy the cell structure of gypenosides to the maximum extent and improve the yield of gypenosides. First, the response surface method was used to optimize the proportion of each enzyme among the complex enzymes, and the optimal combination of the complex enzymes was determined as the content ratio of pectinase, hemicellulase and cellulase was 4:5:5. On this basis, single factor tests (solid-liquid ratio, compound enzyme addition amount, enzymolysis time, enzymolysis pH value, enzymolysis temperature, microwave time) were conducted to explore the influence of each factor on the extraction rate of gypenosides. Secondly, four significant factors were selected for response surface tests to optimize the extraction process. The results showed that the optimal extraction process was as follows: under the condition of 800 W, the amount of complex enzyme was 1.8 %, the enzymolysis temperature was 52 ℃, the enzymolysis time was 2 h, the microwave time was 4 min, the ratio of solid to liquid was 1:30 g/mL, the enzymolysis pH4.8, and the average yield of gynocyanine was 7.88 %.

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Microwave assisted enzyme method is a fast, efficient and simple extraction method, which can play an important role in the extraction of gypenogenin. However, it is necessary to adjust and optimize the specific sample characteristics and experimental conditions to achieve the best extraction effect.

5. New extraction technology

5.1. Continuous dynamic countercurrent method

Continuous countercurrent extraction machine is a continuous flow type extraction device, its principle is similar to the traditional continuous countercurrent extraction, the use of continuous contact and separation between the solid phase and the solution phase to complete the extraction of substances. Specifically, a continuous countercurrent extractor consists of a proportionally mixed mobile phase and a solid phase that flows through a diaphragm pump and is in constant contact with the solid phase to extract the active component in the solid, and then the extract is dissolved in the mobile phase and collected continuously. In the continuous countercurrent extractor, a series of extraction processes can be implemented in a single machine, thus increasing the extraction efficiency.

Yi Kechuan et al. [26] used water as the solvent and a continuous dynamic countercurrent extraction machine to carry out orthogonal tests on the basis of conventional water bath extraction with extraction temperature, extraction time and solid-liquid ratio as parameters. The optimal extraction process was 50 min at the extraction temperature of 80 ℃ and the liquid-solid ratio of 35:1 g/mL. On this basis, the average extraction yield of gypenosides was 8.9%.

As a new type of natural product extraction equipment, continuous countercurrent extraction machine has the advantages of high automation and high extraction efficiency, but its equipment cost is high, a large number of extractants and solids will increase the burden of post-processing, affect the quality of products and other disadvantages. For different application scenarios and requirements, scientific evaluation and reasonable selection are needed.

5.2. Supercritical fluid extraction

Supercritical fluid extraction (SFE) is a new extraction technology with good application prospects in the field. Its principle is to use efficient fluidization characteristics to lock the liquid solvent to the raw material in a supercritical state, and through expansion and rapid cooling processes, the target material can be precipitated and extracted in the shortest time and the smallest dose. Compared with traditional extraction methods, SFE has the characteristics of high precision, environmental protection and high efficiency.

The process of supercritical fluid extraction of gypenoside is as follows: SFE is to apply pressure and temperature to liquid ammonia, acetone, carbon dioxide and other substances, so that it becomes a supercritical fluid. Supercritical fluids have high retention and strong mass transport properties and require accurate contact with solid materials to function. In this process, the supercritical extractant cools and regains circulability. The saponin compounds in supercritical fluid extraction have high solubility and can reach equilibrium quickly. The extracted substance is precipitated by reducing pressure and cooling, and then separated from the extractant to obtain the extracted substance.

Cheng Manhuan et al. [27] used supercritical CO_2 technology to explore the effects of extraction temperature, extraction pressure and extraction time on the yield of gypenosides. Based on the above experimental results, the optimal extraction technology of gypenosides by supercritical CO_2 extraction was determined and compared with traditional organic solvent extraction. The optimal extraction process was as follows: extraction temperature 50 ℃, extraction pressure

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25 MPa, dynamic extraction time 12 min, static extraction time 8 min, CO_2 flow rate 10 ml/min, entraining agent ethanol 1 ml/min, cyclic extraction for three times, the extraction rate of gypenoside was 3.68%. The extraction rate was significantly higher than that of conventional organic solvents (1.97 %).

First of all, it can be extracted at a lower temperature and pressure, avoiding the influence of heat and excessive compression on the active ingredient, making the extract more stable and avoiding the decomposition and degradation of gypenosides. Secondly, carbon dioxide is a non-toxic, odorless and volatile natural substance, which will not remain in the product during the extraction process. In the same case, the extraction cost of supercritical carbon dioxide is lower than that of organic solvents, because it can be recycled and does not need to be treated and cleaned. Finally, the output of the extracted substance is high, and the extraction process is relatively stable, making the product quality more controllable and easier to achieve the requirements of large-scale production. However, because the supercritical state of carbon dioxide requires that the industrial preparation equipment must have high stability and high pressure resistance, the equipment cost is high, which limits the popularity and promotion of this method. Moreover, for plants rich in hard cell membranes, the extraction effect of supercritical carbon dioxide is not as good as that of organic solvents, and the extraction yield and purity are often reduced by the influence of hard cells or components. At the same time, the condensation process is complicated, and the stability and quality of the extract may be reduced if the water and other solutes are not completely removed.

5.3. Ultra-high pressure extraction method

Ultra-high pressure extraction refers to the extraction technology that uses a hydrostatic pressure of 100~1000 MPa to penetrate the extract into the cell at normal temperature, and maintains the corresponding time under the preset pressure to achieve the dissolution balance of the active ingredient, and quickly releases the pressure. Due to the sudden increase in the pressure difference between inside and outside the cell, the active ingredient inside the cell is transferred to the extractant outside the cell, which can improve the extraction efficiency of the active ingredient, shorten the extraction time, and significantly reduce the solvent consumption. Compared with other extraction technologies, the ultra-high pressure extraction technology has higher efficiency.

In one patent [28], the treated raw material was first dried and pulverized, then the mass ratio of 45:1 trichloromethane was added and then reflow was extracted for 3.25h for degreasing operation. Secondly, the aqueous solution of n-butanol was used as the extraction agent, and the ultra-high pressure extraction was carried out under the condition that the ratio of solid to liquid was 1:20, the pressure was 300 MPa, and the pressure was held for 4 min. Then the overpressure extract was purified by AB-8 macroporous adsorption resin. Finally, the extraction rate and purity of gypenosides were 1.13% and 88.72% respectively.

The advantage of ultrahigh pressure extraction is that the process time of ultrahigh pressure extraction is short, and the active components of saponins in gypenoenoides can be efficiently extracted in a short time, and the extract with high purity can be obtained. Do not need any solvent, will not cause pollution to the environment; As a low temperature extraction method, compared with the traditional extraction method, the heat loss and energy consumption are reduced. The ultra-high pressure extraction method is universal and can be applied to the extraction of active components from a variety of plants. However, the high cost of equipment for ultra-high pressure extraction is not suitable for small sample extraction or small-scale laboratory research, and the ultra-high pressure extraction operation is more difficult, requiring a certain level of technology and professional knowledge, and because ultra-high pressure extraction is still in the research and development stage, there is a lack of relevant standardized operating norms and methods.

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5.4. Microbial fermentation method

Microbial fermentation is a method of biological transformation with microorganisms, which produces related enzymes and metabolites through microbial metabolism to extract active ingredients. Microbial fermentation method has certain selectivity, which can adjust the production, activity and generation of metabolites according to the characteristics and physiological state of different microorganisms, so as to improve the extraction efficiency of effective ingredients.

Yang Jingjuan et al., using microbial fermentation method, mixed gypenoylum and excipient bran at the ratio of 3:2, inoculated 5% Trichoderma viridis, cultured at 30 ℃ for five days, and then added 75% ethanol solution to extract at 80 ℃ for 1.5 h. The extraction results were compared with those of conventional reflux method and composite enzyme method. The results showed that the total saponin content of Gypenoenosum extracted by Trichoderma viridis fermentation was 8.34%, compared with the conventional reflux extraction method (5.87%), the extract content was increased by 42.1%, and the extraction effect was better than that of the compound enzyme extraction method (7.69%).

Microorganisms can produce a variety of enzyme systems to play a synergistic role in the fermentation process, so the extraction effect is better than the simple enzyme superposition, which can significantly improve the permeability of cell wall, strengthen the extraction efficiency, and better avoid the influence of heat and solvent factors on the effective components. However, attention should be paid to controlling the growth and metabolic state of microorganisms, and proper adjustment of fermentation substrate and environmental conditions to obtain efficient extraction of gypenosides.

6. Conclusion

With the continuous development of gypenoside extraction technology, there are many different extraction technologies, each of which has its unique advantages and disadvantages. Therefore, according to different raw material characteristics and extraction objectives, it is very important to choose the appropriate extraction technology. At the same time, with the advancement of technology, these technologies are constantly refined and improved to obtain a more efficient, environmentally friendly and economical gypenoside extraction process. Therefore, as a researcher, it is necessary to have a comprehensive understanding and study of different extraction technologies in order to select the optimal extraction scheme, and combine different extraction methods to improve efficiency and improve product quality.

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