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Study on Microwave-assisted Extraction of Gardenia

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ABSTRACT

Plant polysaccharides are compounds that are composed of α-or β-glycosidic bonds by many of the same or different monosaccharides. They are commonly found in natural plants and have molecular weights of tens of thousands or even millions, which constitute life activities. One of the four basic substances, closely related to maintaining the function of life. In recent years, a large number of studies have shown that polysaccharides in addition to immune regulation, anti-tumor biological effects, there are anti-aging, hypoglycemic, anticoagulant and other effects, and its side effects on the body is small, therefore, polysaccharide research has become a medicine Sector of the hot areas. This study aims to study the orthogonal extraction experiment of L9 (33) with water as extractant, and to study the process of microwave extraction of gardenia polysaccharide. Methods: The content of polysaccharides in the samples was determined by phenol - sulfuric acid method. Results: The optimum extraction conditions were as follows: 20 min; solid-liquid ratio: 1: 30; microwave frequency: 2 times, the polysaccharide content was 7.61%

KEYWORDS: plant polysaccharide; gardenia polysaccharide; microwave assisted extraction; orthogonal experiment; extraction process

1. Introduction

Gardenia is a dry ripe fruit of the gardenia jasminoides Ellis, an evergreen shrub, born in the mountains, adaptable, widely distributed in the tropical and subtropical regions, about 250 species around the world. There are four species in China, gardenia, Hainan gardenia, leafy gardenia and spoon leaves gardenia. Gardenia also known as yellow gardenia, mountain gardenia, yellow fruit trees, red gardenia and so on. China's gardenia resources are mainly distributed in Jiangsu and Zhejiang, Anhui, Jiangxi, Taiwan and other southern provinces. Gardenia long oval-shaped, epidermis edge, the shape of the ancient wine. There are five edges, seven edges, nine ribs and other different varieties, in the deep red or yellow red. Bitter cold, non-toxic Into the heart, liver, lung, stomach by. Can heat purging fire, cooling blood. Indications fever sick trouble, jaundice, gonorrhea, diarrhea, red eyes, sore throat, hematemesis, Nvxue, blood dysentery, hematuria, heat sores, sprain swelling and pain. Clinical for acute jaundice hepatitis, bleeding, torsion and other diseases. Modern studies have found that gardenia plants contain many chemical components, such as flavonoids (gardenia), iridoid (geniposide), triterpenes (gardenia), organic acids Esters (chlorogenic acid and safflow acid), also contains D-mannitol, sterols, triterpenoid saponins, long chain alkanes, alcohols and pigments. In the fruit and stems and leaves also contains volatile oil, polysaccharides and other ingredients.

According to the literature, gardenia polysaccharide has a relatively broad spectrum of anti-tumor effect on ascites liver cancer cells and S-180 sarcoma cells have a good inhibitory effect; In addition, there are antipyretic, anti-microbial, calm, liver, gallbladder prevent atherosclerosis and enhance the role of immunity and so on, while the gardenia gardenia polysaccharide is a major component has a role in health care, so valuable to the study of gardenia polysaccharides, in order to take full advantage of gardenia effective polysaccharide In this paper, the extraction conditions of polysaccharides in Gardenia jasminoides will be explored to find out the best technological conditions.

1.1. The purpose and significance of polysaccharide research

Polysaccharide is widely found in nature, is one of the many active ingredients of Chinese herbal medicine, with a variety of biological activity, is the ideal immune enhancer, it can promote T cells, B cells, NK cells, MΦ cells and other immune cells, Can promote the production of interleukin, interferon, tumor necrosis factor and other cytokines. At present, the research on polysaccharide is in the ascendant, the mechanism of action of polysaccharide and the relationship between biological function and structure are deepening, and the new polysaccharide material has been found.

Polysaccharides are monosaccharides linked together by glycosidic chain natural polymer, a polysaccharide was confirmed as scientific information molecule fertilization, development, differentiation, maintenance of nervous system and immune system balance state plays an important role. Scientific research has also confirmed that autoimmune diseases, aging, abnormal proliferation and metastasis of cancer cells, HIV, pathogen infection, a variety of inflammation and other physiological and pathological processes are polysaccharide-mediated. Scientific confirmed covered with an outer sugar chain in a cell of a multicellular organism, such sugar chains involved in cell surface adhesion, formation of branched sugar chain on the cell surface like a radio antenna, which is to pass information between the cells. Polysaccharides on the interpretation of the phenomenon of life provide a very important and reliable basis. To this end, the current international advanced industrial countries have invested heavily in research and development of polysaccharides, polysaccharide research status has become a reflection of a national level of high-tech development of biological scale.

Polysaccharide is the most prominent and universal function is the strengthening of its immune function. Polysaccharides mainly through the following channels to play to promote immune function: (1) to improve macrophage phagocytosis, induced interleukin and tumor necrosis factor production, with the immune function of polysaccharides with lentinan, (2) to promote the proliferation of t cells, induced by the secretion of interleukin, with the immune function of the polysaccharide has such a function of polysaccharides, such as polysaccharides, such as polysaccharides, Polysaccharides, polysaccharides and polysaccharides; (3) to promote the activation of lymphokines; (3) to promote the activation of lymphokines; (3) to promote the activation of lymphokines; killer cell activity, these polysaccharides have wolfberry polysaccharides, APS, Acanthopanax polysaccharide, the bacterium Salmonella typhimurium endotoxin polysaccharide; and (4) increase the activity of b cells, multiple antibodies increase the secretion enhance humoral immune function, These polysaccharides are Tremella polysaccharides, lentinan, fucoidan and alfalfa polysaccharides; (5) through different ways to activate the complement system, some polysaccharides By the alternative complement pathway activation, others by the classical pathway, such polysaccharide zymosan, schizophyllan polysaccharide, polysaccharide angelica, pachyman, Semen polysaccharide, psyllium polysaccharides, bacterial lipopolysaccharide, lentinan like.

Polysaccharides have a pivotal role in human health, it can control cell division and differentiation, regulating cell growth and aging. It also has the function of regulating immunity, is now known as the best immune enhancer, accelerator and regulator, immune disorders caused by the widespread use of the disease, it can not only prevent cancer on the immune system, serious damage, but also Prevention and treatment of a variety of diseases due to immunodeficiency and chronic viral hepatitis, recurrent respiratory disease, viral influenza and caused by drug-resistant bacteria caused by long-term treatment of tuberculosis, clinically proven polysaccharides can also significantly prevent streptomycin-rifampicin treatment of tuberculosis The recurrence.

In the field of life science research, sugar biology has always been the frontier and hotspot in this field. Recent studies have shown that the major cell adhesion molecules involved in cell-to-cell, cell-matrix interactions are mostly glycoconjugates, although the role of sugar chains in cell recognition and adhesion molecules is still not fully elucidated , But the importance of these cell adhesion molecules in the body and medicinal potential has shown great appetite. Such as 1990 found E-Selectin, the vascular endothelial cell - leukocyte adhesion molecule, can identify the surface of the white blood cell sialylated tetrasaccharide molecule SLex, when the tissue is damaged or infected, the white blood cells and endothelial cells adhesion, and then rolling along the wall Through the vessel wall into the damaged tissue to kill the invading pathogens. Another example is the transfer of cancer cells, pathogens such as infection process is also mediated by glycoproteins, and sugar chain involved in its identification and adhesion. Abroad has been reported through certain peptides or carbohydrate compounds to block the transfer of experimental lung cancer, made some promising progress, of course, to achieve clinical applications are still a large distance. In recent 20 years, a large number of polysaccharides and sugar conjugates from Chinese herbal medicine, such as Astragalus polysaccharides, Achyranthes bidentata polysaccharides, Polyporus umbellatus polysaccharides and medlar candy candies, have been chemically and extensively studied. These polysaccharides and sugar conjugates have immunomodulatory, anti-tumor, hypoglycemic and other aspects of pharmacological effects, and some have been in clinical applications.

1.2. Status and Analysis of Domestic (Foreign) Research

As early as the twentieth century, polysaccharides began to serve as drugs, and to the sixties, because of its wide range of immune promoters caused great interest in the development of polysaccharide health food and drugs also pay much attention. Polysaccharides are not only medicinal in many ways, but also very toxic as a drug. The research on the physiological activity and extraction and purification of polysaccharides from different sources has achieved great success. At present, the mechanism of action and structure-activity relationship of active polysaccharides is being studied at home and abroad, and some progress has been made.

The past two decades, due to the development of molecular biology, it is increasingly recognized and sugar complex molecule having extremely important biological functions, regulation identification polysaccharide immune function, cell-cell, intercellular transport of substances and cancer Diagnosis and treatment, have a close relationship.

Polysaccharides are still a good adjuvant in medicine. In recent years, polysaccharides have been found in the sugar chain in molecular biology has a decisive role. In addition it can control cell division and differentiation, regulate cell growth and aging. Polysaccharides in the food industry, fermentation industry and the oil industry also has a wide range of applications. Therefore, in the development of polysaccharide resources, polysaccharide structure analysis, polysaccharide pharmacological effects of research, people do a lot of work. Polysaccharides are derived from higher plants, animal cell membranes, microbial cell wall in the natural macromolecules, is an important part of all living organisms, and maintenance of life required for a variety of physiological functions. However, in terms of its research situation, although the saccharide has made great progress, but with the protein and nucleic acid leap-like development, it is far behind. The study of polysaccharides was first carried out in the 1940s, and the earlier and most studied ones were obtained from bacteria in various capsular polysaccharides, which were used primarily in medicine for vaccines. However, polysaccharides as a broad-spectrum immune accelerator and cause great attention is in the 20th century, 60 years. Especially in the past two decades, due to the development of molecular biology, people have come to realize that sugar and its complex molecules have extremely important biological function, and have held many times on the 'sugar biology and sugar engineering' In 1984, the Soviet Union in the Netherlands held at the twelfth International Carbohydrate Symposium reported the use of synthetic specific structure of the capsular polysaccharide as a vaccine, by the participants of great interest. Later, the research on fungal polysaccharides is more extensive and extensive, such as yeast polysaccharides, edible fungi polysaccharides, especially edible fungus polysaccharide research, reported a higher number, of which lentinan research more clearly. Japan will be mushroom polysaccharide, Yunzhi polysaccharide: ps-k into the market, and achieved significant benefits. In March 1995, the Asian Molecular Biology Organization (ambo) also held a 'sugar biology and sugar engineering' training course in Japan, that is, 'the era of sugar biology is accelerating.' Polysaccharides have the storage energy, structural support and antigenic decisiveness and other biological functions, polysaccharide and sugar conjugate separation, purification and component determination and structural analysis has made great progress. At the same time, the biological function of the polysaccharide has a new understanding.

Since the 1980s, scientists have been interested in plant polysaccharides, especially polysaccharides in traditional Chinese medicine, and have reported more than 100 kinds of immunoregulation, anti-tumor, anti-virus, anti-infective, hypoglycemic and other Physiological activity of traditional Chinese medicine polysaccharide, and some have been used in clinical clinical use of cancer, hepatitis, cardiovascular and other diseases of the adjuvant therapy and rehabilitation, one of the most important pharmacological effects when pushing immunization effect. There are a large number of pharmacological and clinical application shows that these functions of the exact polysaccharide, most of its original drugs are tonic Chinese medicine, such as ginseng polysaccharides, Astragalus polysaccharides of the original drug ginseng, astragalus are well-known Chinese medicine; Angelica polysaccharide, (Achyranthan, Abps), recent pharmacological studies and clinical applications have shown that it has a significant immune enhancement, and its original drug Achyranthes bidentata Is a 'strong bones, governance Yaoxishen Ma' effect of traditional Chinese medicine, and these effects are all immune function. So far the majority of traditional Chinese medicine immunomodulator is immune accelerator, and its active ingredient is polysaccharide or sugar conjugate, including glycopeptide, proteoglycans and so on.

China's natural plant polysaccharide extraction technology - from a variety of Chinese herbal medicine to extract the purity of active polysaccharides has reached more than 98%, which is China's important areas in the life sciences, 'active polysaccharide' separation, purification, molecular structure and quantity Efficiency relationship control made a major breakthrough, into the forefront of life science to reach the world leading level.

In particular, the medical community is concerned that polysaccharides can activate macrophages, T lymphocytes and B cells, to strengthen the antibody, to inhibit and eliminate the effect of tumor cells. Polysaccharides can also promote the differentiation of insulin to support the amount of glycogen, polysaccharides absorbed by the body, only a very small amount of insulin to participate, will not increase the burden of insulin. Therefore, polysaccharides can prevent diabetes complications, in 1990, Japan specifically reported polysaccharides can be used as eye disease control of the disease, can prevent and cure diabetic retinal rupture; polysaccharide can significantly reduce blood lipids, cholesterol, blood pressure.

Polysaccharides have the activity and immunity and rarely have side effects, is the ideal natural, safe and effective drugs and health food products, showing a bright field for human health, for which scientists called the 21st century polysaccharide century.

At present, at least 12 kinds of polysaccharides are used as antineoplastic drugs in clinical trials. In recent years, polysaccharides have been found in the sugar chain in molecular biology has a decisive role. In addition, polysaccharides can also control cell division and differentiation, regulate cell growth and aging. Polysaccharides in the food industry, fermentation industry and the oil industry also has a wide range of applications. Therefore, in the development of polysaccharide resources, polysaccharide structure analysis, polysaccharide pharmacological effects of research, people do a lot of work. At present, polysaccharides research to Japan, the United States, Germany, Russia and other countries in a leading position, our scientists have done a lot of work. In particular, plant polysaccharides, because

China is the origin of traditional Chinese medicine, and sugar is a common ingredient in Chinese herbal medicine, in a variety of Chinese herbal medicine chemical composition of the process, people are ultimately concerned about the polysaccharide. At present, the research on the mechanism of action and structure-activity relationship of active polysaccharides at home and abroad. With the people's awareness of environmental protection, return to nature has become a daily life in the pursuit. The concept of sub-health status, the introduction of the state's reform of the health care system and the improvement of living standards have promoted the transformation of people in the concept, that is, from the past to the concept of disease-based gradually to the concept of disease prevention, This provides a broad prospect for the development and application of natural polysaccharides. Traditional Chinese medicine is the treasure house of medicine in our country. Modernization of traditional Chinese medicine is the only way to the development of traditional Chinese medicine. The research of natural polysaccharide has made remarkable progress both in pharmacy and pharmacology. Its in-depth research and development will become a modern medicine model.

2. Experimental part

2.1. Experimental raw materials, equipment and pharmaceuticals

2.1.1 Experimental raw materials Gardenia Gardenia (Xinjiang Shihezi City Drugstore)

2.1.2 Experimental instrument 722N visible spectrophotometer, grinder, electric sets, one thousandth of a balance, circulating water type multi-purpose vacuum pump, electric heating oven, microwave extractor (Matsushita Electric), filter, Bottle, measuring device, all kinds of pipettes (various specifications), separatory funnel, Erlenmeyer flask, filter device.

2.1.3 Experimental drugs Glucose (AR, 105 dry to constant weight), phenol (RA), concentrated sulfuric acid (RA)

Ethanol (95%), petroleum ether, ethyl acetate, acetone

2.2. Experimental methods and procedures

2.2.1 Determination of microwave extraction conditions

2.2.1.1 Effects of Microwave Time on Extraction of Gardenia

Control the ammeter reading in the 60-80mA, water and gardenia liquid to solid ratio of 10: 1, microwave once, the application of different microwave treatment time, the extraction of gardenia polysaccharides, the results shown in Figure 1

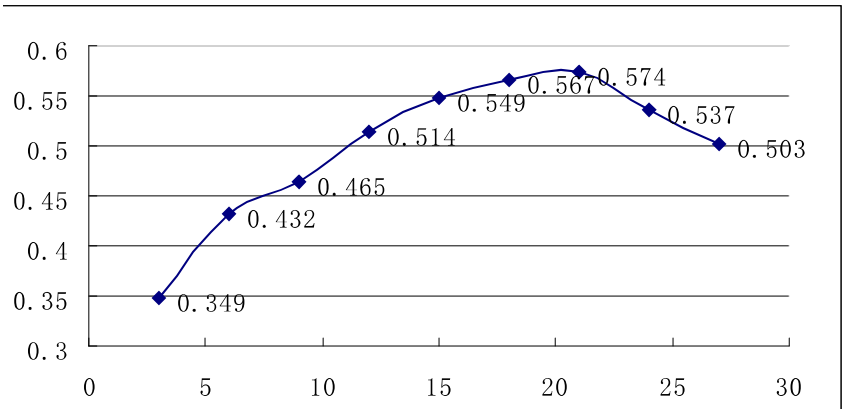

Figure 1. Relationship between microwave heating time and absorbance of gardenia polysaccharide solution

It can be seen from Figure 1, microwave heating time ≤ 21min, with the microwave heating time, gardenia polysaccharide solution absorbance increased; but the heating time ≥ 21min, with the heating time, Gardenia polysaccharide absorbance decreased. This may be due to the fact that the microwave treatment for a long time produces too high a temperature and causes the destruction of the polysaccharide. It can be seen from the absorbance, microwave heating 21min gardenia polysaccharide content is higher.

2.2.1.2 Effects of microwave frequency on the extraction of gardenia polysaccharide

Control the ammeter reading in the 60-80mA, water and gardenia liquid to solid ratio of 10: 1, microwave heating time of 20min, the use of microwave treatment of different times, the extraction of gardenia polysaccharide research, the results shown in Figure 2.

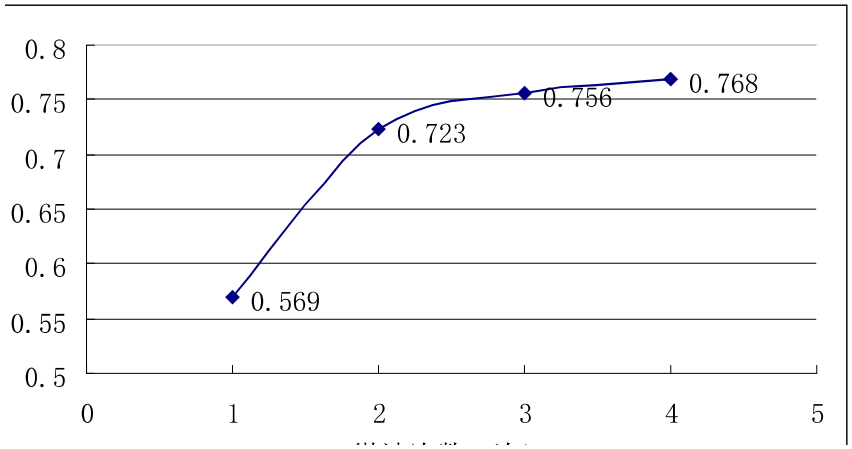

Figure 2. Relationship between microwave frequency and absorbance of gardenia polysaccharide solution

It can be seen from Figure 2, with the increase in the number of microwave, gardenia polysaccharide solution absorbance gradually increased, but the microwave 3 times after the increase is not obvious. Considering the cost, the microwave frequency should be 2-3 times.

2.2.1.3 Effect of water and weight ratio of water to gardenia on the extraction of gardenia polysaccharide

Control the ammeter reading at 60-80mA, microwave heating time of 20min, microwave 2 times, to study the different liquid-solid weight ratio of gardenia polysaccharide extraction, the results shown in Figure 3.

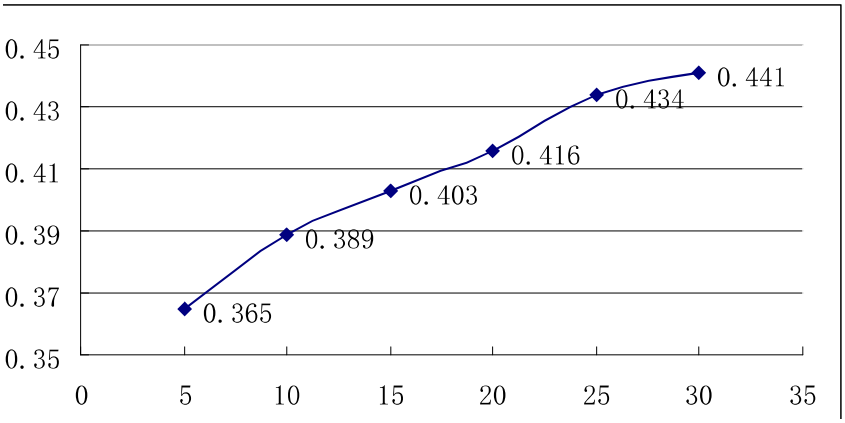

Figure 3. Relationship between liquid-solid weight ratio and absorbance of gardenia polysaccharide solution

It can be seen from Figure 3, in a certain range, the greater the liquid-solid weight ratio, gardenia polysaccharide solution absorbance is greater, liquid-solid weight ratio ≥ 25: 1, the increase is not obvious. Considering the cost, the liquid to solid weight ratio is in the range of 25: 1-30: 1.

2.2.2 Preparation of standard curves

Glucose standard solution preparation: accurately weighed dry constant weight glucose 25.2mg, add appropriate amount of water dissolved, transferred to the 250ml volumetric flask, add water to the scale shake, with 100.8μg / ml standard glucose solution.

Preparation of 5% phenol standard solution 100g of phenol, 0.1g of aluminum and 0.05g of NaHCO3, distillation, 182 a large number of fractions. Quickly weighed 7.5g fractions, add appropriate amount of water prepared into 5% phenol solution, placed in a dark bottle into the refrigerator for spare.

Preparation of standard curve Accurately measured glucose standard solution 0,0.1,0.2,0.3,0.4,0.5,0.6,0.7ml placed in a dry large test tube, respectively, add water to 1ml, then add 5% phenol 1ml, shake , Then concentrated H2SO4 5.0ml, shake well, room temperature for 30min to room temperature, measured at 490nm maximum absorbance value.

No.	1	2	3	4	5	6	7
V (ml)	0.1	0.2	0.3	0.4	0.5	0.6	0.7
C (ug/ml)	10.08	20.16	30.24	40.32	50.40	60.48	70.56
A	0.078	0.163	0.245	0.289	0.365	0.438	0.547

Data processing regression equation: A = 0.0074C + 0.0069 (r = 0.9915)

Glucose standard curve

2.2.3 Determination of conversion factors

Gardenia crushed, weighed 100g, placed in a conical flask, followed by 250ml petroleum ether, ether and 80% ethanol ultrasonic extraction twice, the reaction time of 20min, decompression pumping, residue residue after the solvent, continue to Water ultrasonic extraction twice, 40min / time, under reduced pressure filtration, combined filtrate, the filtrate was concentrated to half the volume, adding 0.1% activated carbon, decolorization twice, filtration. The filtrate plus 4 times the amount of 95% ethanol, placed overnight, after pumping the precipitate in 100ml water dissolved, repeated precipitation once, filter, the residue with ether, ethanol repeatedly washed. 60 ℃ dry, that was gardenia polysaccharide.

Accurately weighed 60 dry constant weight of gardenia polysaccharide 20.00μg, dissolved in water to 100ml volumetric flask, shake as a polysaccharide stock solution. Accurately measure the polysaccharide stock solution 0.2ml, add water to 1ml. The absorbance was measured in the same manner as in the standard curve. The conversion factor A = 0.295 was calculated as follows:

$$F = W / CD$$

Where W is the polysaccharide weight (μg), C is the concentration of glucose in the polysaccharide solution (μg / ml), and D is the dilution factor of polysaccharide.

2.2.4 Design of orthogonal experiments

Table 1. Factor Level List

Factor	Factor A microwave time(min)	B microwave frequency	C solid-liquid ratio (g/ml)
1	15	1	1:20
2	20	2	1:25
3	25	3	1:30

According to this orthogonal experiment, the extraction process of polysaccharides in gardenia will be studied.

2.2.5 Extraction of gardenia polysaccharide

Weigh a certain amount of crushed gardenia, according to the previously designed orthogonal experiment, designed as shown in Table 2, the nine groups of experiments.

Accurately weighed 9 copies of a certain amount of gardenia powder, placed in the flask, with 95% ethanol soaking, numbered 1,2,3,4,5,6,7,8,9. Respectively, into the microwave extractor, with a certain amount of 95% ethanol reflux 15min, control current 60-80mA. Filter, extractor and then a certain amount of ethanol reflux 15min, control current 60-80mA. Filter, full washing, the filtrate moved to 500ml volumetric flask, constant volume, shake. And then diluted a certain number of times to measure the standard curve of the same method to measure the absorbance A, according to the following formula to calculate the polysaccharide content:

$$\text{Polysaccharide content (\%)} = CDf / W \times 100$$

Where C is the concentration of glucose in the sample solution (μg / ml), D is the dilution factor of polysaccharide, f is the conversion factor, and W is the weight of the sample (μg).

Experiment No.	A	B	C	Polysaccharide content (%)
1	1	1	1	5. 67
2	1	2	2	6. 25
3	1	3	3	6. 91
4	2	1	2	6. 72
5	2	2	3	7. 61
6	2	3	1	6. 03
7	3	1	3	5. 72
8	3	2	1	5. 65
9	3	3	2	5. 64
K_1	18. 83	18. 11	17. 35	
K_2	20. 36	19. 58	18. 61	
K_3	17. 01	18. 58	20. 24	
k_1	6. 28	6. 04	5. 78	
k_2	6. 79	6. 53	6. 20	
k_3	5. 67	6. 19	6. 74	
Difference	1. 12	0. 49	0. 96	
Factor primary and secondary	I	III	II	
Superior level	6. 79	6. 53	6. 74	

Table 2. (L933) Orthogonal Experiment Best Conditions List

3. 3 Results and discussion

The optimum extraction process of gardenia polysaccharide was A2B2C3, that is, microwave assist time was 20min, microwave frequency was 2 times, solid-liquid ratio was 1:30. The order of influence on the content of polysaccharides in gardenia was A> C> B. There was no significant difference between the factors.

There are many ways to extract Chinese herbal medicine, but the commonly used extraction methods (such as boiling method, reflux method, impregnation method and percolation method) to retain the active ingredient, remove the invalid ingredients, there is a large loss of active ingredients, Process, extraction rate is not high and other shortcomings. In the past 10 years, there have been many new technologies and methods in the extraction of traditional Chinese medicine. The application of these new technologies and methods has made the extraction of Chinese herbal medicine both in line with the traditional theory of traditional Chinese medicine and the purpose of improving the yield and purity of active ingredients. Such as: supercritical fluid extraction technology, ultrasonic extraction technology, microwave extraction technology, enzymatic, semi-bionic extraction method, broken extraction and so on. The use of microwave

extraction technology is mainly due to microwave extraction process and the traditional extraction process has the following advantages:

1. Can be selective to the role of energy on the active ingredients of the material, improve product purity.
2. Equipment, small size, low cost.
3. Low energy consumption, only 6 times the power consumption of home microwave oven.
4. Reduce the operating steps, shorten the production time.
5. To reduce waste emissions.
6. Heating and control the output of energy quickly.

Conclusion

At present, microwave extraction in the processing of agricultural products, Chinese medicine purification, spice extraction has made great economic benefits. It is no exaggeration to say that microwave extraction (extraction) for the majority of chemical, pharmaceutical, agricultural high-tech enterprises and technology workers to open up a broad potential market.

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