



AN ANTI-TUMOR CHINESE MEDICINE PREPARATION COMPRISING LUCIDUM SPORES OIL AND PREPARATION METHOD THEREOF

5 FIELD OF INVENTION

The present invention relates to pharmacy field, in particular, relates to an anti-tumor Chinese medicine preparation comprising Lucidum spores oil and preparation method thereof.

10 BACKGROUND OF THE INVENTION

Malignant tumors seriously threaten human health. In recent years, the incidence of malignant tumors has increased year by year. The cancer incidence and mortality have occupied the first place of single tumor disease in China or even worldwide. Because
15 the mechanism of the malignant tumor occurrence and development is unclear in modern science, there is no method which can achieve the purpose of curing malignant tumors.

The main methods for treating malignant tumors of the western medicine include surgery, radiotherapy and chemotherapy.

20 The surgical treatment is used for limited tumors, which has radical treatment effects. The radical resection operation assisted by regional lymphograph can relatively integrallty resect the tumors and clear the surrounding possible lesions. The postoperative specimens can be used for pathological examination to confirm the tumor type, metastasis and whether the resection is complete, which has certain
25 reference value for the next treatment. However, under normal circumstances, patients with malignant tumor have micro-metastasis during the operation or before the operation, and there are a certain amount of tumor cells after operation in patients. For



example, if the lung adenocarcinoma is 1cm long in diameter, there are about 30% distant metastasis, and if 2cm long, there are about 60% metastasis. Therefore, most patients can not prevent tumor recurrence and distant metastasis alone with surgery, and the radical treatment efficacy still can not be obtained even superradical operation is made. Some patients has lost the operative indications when diagnosed. In addition, the operation has certain interference to the the physiological function of patients, especially the damage to the immune function, thus affecting the surgical results and prognosis.

The post-operative radiotherapy and chemotherapy can kill a certain number of remaining cancer cells and reduce the tumor recurrence.

(1) Radiation therapy uses radiation to kill cancer cells, which is suitable for the limited radiation of sensitive tumor and generally used to treat the primary foci, sub-clinical foci and lymph node metastasis of tumors. But radiotherapy also has damage to the normal tissue in the radiation zone, thus producing systemic and local reactions, so the radiotherapy is not suitable for patients with distant metastasis. In addition, some tumors are not sensitive to radiation, so radiotherapy has some limitations and should be combined with other treatment methods. (2) In the last decade, chemotherapy has been rapidly developed, especially the treatments for leukemia and malignant lymphoma have obtained good results, and the development of new anti-tumor chemotherapy drugs, great progress in combination chemotherapy and application of combination therapy improve the efficacy of cancer chemotherapy. But there are many disadvantages of chemotherapy. Theoretically, the chemotherapy kills the cancer cells in logarithmic degree and can not kill all cancer cells in vivo, and there are about $10^6 \sim 10^8$ cancer cells remained; and most anti-tumor chemotherapy drugs are short of desired selectivity, which can inhibit tumor cells and also damage the normal cells, thus causing severe adverse reaction of tumor patients and damage of hematopoietic system, digestive system, nervous system, liver and kidney function to force the discontinuing of patients.

(2) Modern TCM oncologists believe that the diagnosis of tumor and the time of using regular treatment (mainly surgery, radiotherapy and chemotherapy) have significant impact on the prognosis of tumor. In the promotion of early surgery, the overall condition of patient before surgery should be noted. Many scholars believe that the application of TCM during this stage should be based on regulating qi, blood, yin and yang and zang-fu function and make the patients recover close to the state of "yin and yang in relative equilibrium", which is the key to the successful completion of surgery, radiotherapy and chemotherapy. The methods for supplementing qi, nourishing yin and regenerating body fluid are commonly used to achieve the effects of obviously relieving the clinical symptoms of cancer patients after radiotherapy, protecting the hematopoietic system function, improving immune function, reducing tissue damage, decreasing toxicity and increasing efficiency and inhibiting tumors, thus the radiotherapy can be successfully implemented. In modern times most of TCM scholars believe that the pathogenesis of adverse reactions in patients after chemotherapy are mainly qi and blood damage, impairment of yin caused by heat toxin, disorders of the spleen and stomach and so on. Currently the treating principles of chemotherapy reaction are supporting the healthy energy and using the methods for replenishing qi and blood and nourishing liver and kidney, which can often significantly reduce a variety of clinical symptoms in patients with tumors after chemotherapy, prevent the decline in white blood cells and platelets in the process of chemotherapy, regulate immune function, significantly improve the completion rate of chemotherapy treatment in patients with tumors, improve the survival quality of patients with its effects of decreasing toxicity, increasing efficacy and anti-tumor, and prolong the survival time. The treatment after surgery and radiotherapy and chemotherapy mainly adopted to restore the body function, and inhibit and eliminate the remained cancer cells to consolidate the effects and prevent the recurrence and metastasis. The treatment mainly aimed at common symptoms, such as fever, night sweats, fatigue, loss of appetite and other symptoms, to carry out an overall adjustment. For the cancer patients who had lost the best timing of surgery or radiotherapy and chemotherapy, based on the the patient's specific situation, we can give priority to the support of the healthy energy, and simultaneously eliminate and reinforce to achieve the purpose of improving the life quality and prolonging the life.

In short, the treatment method with surgery, radiotherapy, chemotherapy as the main methods used by western medicine was the main measures to treat malignant tumors, but the efficacy of considerable number of patients was not satisfactory. When the cancer patients were administrated with western medicine, we can use Chinese medicine treatment based on supporting the health energy at the same time, which has distinct advantages of anti-tumore, decreasing toxicity, increasing efficacy and reducing adverse reactions. It is necessary and possible to take Chinese medicine theory as guidance, take clinical efficacy as a basis, aim at the occurrence and development of tumors and the pathogen of body damage caused by western medicine therapy, develop the drugs for preventing and curing malignant tumors with the purpose of prevention, early treatment and searching for the primary cause of disease in treatment from the perspective of regulating the overall function.

15 SUMMARY OF INVENTION

The objective of the present invention is to provide an anti-tumor Chinese medicine preparation containing *Lucidum* spores oil, which has effects of regulating immune function, anti-cancer, anti-tumor, reducing side effects of western modern medicine and relieving symptoms, and is applied before and after surgery and radiotherapy and chemotherapy and is suitable for malignant tumor patients with qi and yin deficiency who could not undergo the surgery or radiotherapy and chemotherapy. The present invention also provides a preparation method for said Chinese medicine preparation.

The present invention provides an anti-tumor Chinese medicine preparation containing *Lucidum* spores oil. The raw materials of said preparation are *Lucidum* spores and *Atractylodes* rhizome.

The ratio of *Lucidum* spores and *Atractylodes* rhizome by weight in the Chinese

medicine preparation is preferably 1:1.

The above raw materials are combined with pharmaceutically acceptable excipient to prepare into various pharmaceutical oral dosage forms.

The present invention also provides a preparation method for the Chinese medicine preparation, the specific steps of which are as follows:

a: respectively cleaned *Lucidum* spores and *Atractylodes* rhizome, and respectively weighed the cleaned *Lucidum* spores and *Atractylodes* rhizome according to the weight ratio;

b: broke the *Lucidum* spores obtained from the step a through wall-breaking and grinding;

c: broken *Lucidum* spores obtained from the step b are granulated and dried to give dry *Lucidum* spores particles;

d: the dry *Lucidum* spores particles obtained from the step c are mixed with *Atractylodes* rhizome obtained from the step a, and the mixture is carried out extraction of crude oil mixture and removal of impurities and moisture to give the mixture oil of *Lucidum* spores and *Atractylodes* rhizome, namely the above mentioned Chinese medicine preparation.

In the above preparation method, a pulverizer for wall-breaking and grinding can be used in the step b to broke the *Lucidum* spores. The service conditions of the cell disruption pulverizer are as follows: the content of the *Lucidum* spores are 1 to 3kg, the time for breaking the cell wall each time is 1 to 2h, preferably 2kg of the content, 1.5 h of each time for breaking the cell wall.

The preferred condition for granulation in the step c are as follows: the broken Lucidum spores are added with distilled water in an amount of 20-30% by weight of the broken Lucidum spores, mixed and prepared into soft material, and then the soft materials are placed into a granulator and granulated with a 10 mesh stainless steel
5 sieve to give the Lucidum spores particles; the preferred drying condition in the step c are as follows: the above mentioned Lucidum spores particles are placed into a hot air cycle oven and dried at the temperature of lower or equal to 60°C. The addition amount of the distilled water is preferably 25% of broken Lucidum spores weight.

In order to efficiently extract oil mixture of Lucidum spores and Atractylodes
10 rhizome, the method for extracting the oil mixture in the step d is preferably CO₂ super critical extraction, and the extraction conditions are as follows: the extraction temperature is 40 to 60°C, the pressure is 18 to 26 MPa, CO₂ flow rate is 0.4 to 0.6m³/h, and the extraction time is 3 to 5 h, preferably extraction temperature of 60°C, pressure of 26 MPa, CO₂ flow rate of 0.6m³/h and extraction time of 5 h.

15 The oil mixture of Lucidum spores is prepared into soft capsules through conventional ways.

The oil mixture of Lucidum spores and Atractylodes rhizome is included by β -cyclodextrin to obtain the clathrate compound of Lucidum spores and Atractylodes rhizome oil mixture.

20 The preferred clathration method is as follows: 4-8 parts by weight of β -cyclodextrin and 2-4 parts by weight of water are well mixed, added with 1 part by weight of Lucidum spores and Atractylodes oil mixture, ground for 20-40 min and stored under low temperature followed by air pump filtration, then the mixture is washed with 0.1-0.3 parts by weight of anhydrous ethanol and dried under vacuum to obtain the
25 clathrate compound of Lucidum spores and Atractylodes rhizome oil mixture.

The above clathrate compound is prepared into tablet or hard capsule.

The present invention provides an anti-tumor Chinese medicine preparation containing Lucidum spores oil, which has effects of regulating immune function, anti-cancer, anti-tumor, reducing side effects of western modern medicine and relieving symptoms, and is applied before and after surgery and radiotherapy and chemotherapy and is suitable for malignant tumor patients with qi and yin deficiency who could not undergo the surgery or radiotherapy and chemotherapy. Lucidum spores have sweet and mild nature and effects of treating consumptive diseases, replenishing essence qi and supporting the healthy energy, which are germ cells of Lucidum. Atractylodes rhizome has warm nature and sweet and bitter flavor, which has effects of reinforcing spleen and stomach and eliminating dampness and calming fetus. The preparation only has two medicines and the nature is mild. These two medicines are combined to give the effects of supplementing qi and nourishing yin and supporting the healthy energy. When applied in patients with tumors, the preparation has effects of strengthening the physical fitness of patients, replenishing the weak state, adjusting the body internal environment and inhibiting the growth of tumor cells. In recent years, the chemical composition and medicine efficacy of Lucidum spores and Atractylodes rhizome have been carried out with deep study. Lucidum spores have protein, amino acids, polysaccharide glycopeptides, adenosine, steroids, triterpenoids, alkaloids, fatty acids and other chemical substances. Pharmacological studies have shown that Lucidum spores have effects of anti-chromosomal mutation, inducing tumor cell apoptosis, inhibiting tumor cell proliferation, killing cancer cells, antagonizing the adverse reactions caused by cyclophosphamide, such as decreased endurance in mice, chromosomal aberrations, decreased hemoglobin and white blood cells, decreased macrophage phagocytic activity and increased SGPT, inhibiting the ^{60}Co irradiation-induced adverse reaction of leukopenia, activating and improving the anti-tumor effects of specific killer cells (CTL) and non-specific killer cells (NK, LAK), enhancing the phagocytic function of monocyte-macrophage, and anti-lipid peroxidation, which indicates that Lucidum spores have good application prospects in regulating the immune function and anti-

- cancer. The volatile oil of *Atractylodes* rhizome has inhibition effects on the mouse sarcoma S₁₈₀, ehrlich ascites carcinoma and ascitic lymphosarcoma, and the inhibition rate on mice sarcoma S₁₈₀ is 31%-49%. The volatile oil of *Atractylodes* rhizome also has significant inhibition effects on cultured human esophageal carcinoma cell line.
- 5 The tumor growth inhibition mechanism of *Atractylodes* rhizome is related with decreasing the proliferation rate of tumor cells, reducing the invasiveness of tumor tissue and improving the body's reaction ability of anti-tumor and the cytotoxicity on tumor cells. Chinese medicine theory, practical experiences and pharmacology study results suggest that this prescription has effects of regulating immune function, anti-
 - 10 tumor and anti-cancer, reducing the adverse reactions of western medicine, relieving symptoms and other effects, which is applied before and after surgery and radiotherapy and chemotherapy and is suitable for malignant tumor patients with qi and yin deficiency who could not undergo the surgery or radiotherapy and chemotherapy.
 - 15 The preparation method of said Chinese medicine provided by the present invention can efficiently extract the oil mixture of *Lucidum* spores and *Atractylodes* rhizome. After the oil mixture is included with β -cyclodextrin, the chemical compositions of the *Lucidum* spores and *Atractylodes* rhizome oil mixture has no change, and the solubility after made into the clathrate compound is significantly increased, thus
 - 20 improving the bioavailability of essential oil, reducing the irritation and side effects of volatile oil, covering up their bad smell, greatly improving the stability of volatile oil, reducing the dispersion or oxidation deterioration of the volatile oil in the process of production and storage, enhancing the stability of the preparation, improving the quality of this product. The volatile oil liquid drug is prepared into solid and powder
 - 25 to facilitate a variety of dosage forms, such as tablets, hard capsules, etc. with easy medicine-taking and portability.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the preparation process of anti-tumor Chinese medicine preparation

containing Lucidum spores oil.

Figure 2 shows the UV characteristics diagram of each substance, with the absorbency as the vertical coordinate and the absorption wavelength as the abscissa, in which 1 represents oil mixture of Lucidum spores and Atractylodes rhizome; 2 represents physical mixture of Lucidum spores and Atractylodes rhizome oil mixture with β -cyclodextrin; 3 represents materials after de-clathration; 4 represents clathrate compound; and 5 represents β -cyclodextrin.

DETAILED DESCRIPTION OF THE INVENTION

- 10 Embodiment 1 Method for preparing anti-tumore Chinese medicine preparation containing Lucidum spores oil - oil mixture of Lucidum spores and Atractylodes rhizome

The preparation process of the Chinese medicine preparation is as follows (Figure 1):

- a: respectively cleaned Lucidum spores and Atractylodes rhizome, and respectively
15 weighed the cleaned Lucidum spores and Atractylodes rhizome according to the weight ratio;

The so-called cleaning means eliminating the impurities in the Lucidum spores and Atractylodes rhizome, and then the cleaned Lucidum spores and Atractylodes rhizome were respectively weighed according to the weight ratio.

- 20 b: broke the Lucidum spores obtained from the step a;

Bailey pulverizer for walk-breaking and grinding (BFM-100A type, Jinan Beili Powder Technology Engineering Co., Ltd.) was used to break the cleaned Lucidum

spores with water as the cooling medium with the pressure of more than 0.65MPa, the flow rate of 3M³/min, the cooling medium temperature of less than 20°C, grinding chamber temperature of less than 50°C, the spindle speed of 970/min, and 500 friction rods.

- 5 Determination of the feeding quantity and time in the wall-breaking and grinding process: Lucidum spores were taken followed by wall-breaking and grinding, and according to the features of the wall-breaking and grinding device, under the conditions of each fixed parameter, such as spindle speed, friction rod, cooling medium and cooling time (1h), different feeding quantities from 1 to 3kg were
- 10 selected to carry out the experiment with the powder yield and the wall-breaking rate as test indexes. The results showed that the feeding quantity had little influence on the powder yield; the wall-breaking rate was inversely proportional to the feeding quantity. Considering the production cost and efficiency, the feeding quantity was 2kg (see Table 1). After the feeding quantity was confirmed as 2kg, the wall-breaking and
- 15 grinding time was selected (see Table 2) and three batches of validation should be in accordance with the test results (see Table 3).

Serial No.	Weight before wall-breaking (kg)	Weight after wall-breaking (kg)	Powder yield (%)	Wall-breaking rate (%)
1	1	0.96	96	≥95
2	1.5	1.4	93	≥95
3	2	1.92	96	≥95
4	2.5	2.34	94	≥90
5	3	2.82	94	≥60

Table 1: Test datasheet of Lucidum spores feeding quantity for wall-breaking

Serial No.	Wall-breaking and grinding time(h)	Weight before wall-breaking (kg)	Weight after wall-breaking (kg)	Powder yield (%)	Wall-breaking rate (%)
1	1	2	1.92	96	≥ 90
2	1.5	2	1.92	96	≥ 95
3	2	2	1.92	96	≥ 95

Table 2: Test datasheet of Lucidum spores wall-breaking time

Serial No.	Weight before wall-breaking (kg)	Wall-breaking and grinding time (h)	Weight after wall-breaking (kg)	Powder yield (%)	Wall-breaking rate (%)
1	2	1.5	1.94	97	≥ 95
2	2	1.5	1.92	96	≥ 95
3	2	1.5	1.92	96	≥ 95

Table 3: Test datasheet for wall-breaking validation of three batches of Lucidum spores

Therefore, when using Bailey pulverizer for wall-breaking and grinding, the feeding quantity of Lucidum spores was 2kg, and each wall-breaking and grinding time was 1.5h. The wall-broken Lucidum spores powder was determined with scanning electron microscope and when the particle size was smaller than or equal to $5\mu\text{m}$, the wall-breaking rate was greater than or equal to 95%. The normal distribution was determined as $90\% \leq 5\mu\text{m}$.

c: broken Lucidum spores obtained from the step b were granulated and dried to give dry Lucidum spores particles.

(I) Granulation

15 Study on the water addition of soft materials: In order to facilitate granulation, the

5 wall-broken Lucidum spores were prepared into soft materials. Considering that the surface energy of wall-broken Lucidum spores was relatively large, water (distilled water) was selected as wetting agent. The water addition was investigated through visual observation, and the test results showed that the obtained soft material was better when the weight ratio of wall-broken Lucidum spores and water was 1 : 0.25 (Table 4).

Serial No. Lucidum spores (kg)	Distilled water (ml)	Situation of soft material
11	300	Soft materials were sticky
21	250	Soft materials had good looseness
31	200	Soft materials had many fine powders

Table 4 :Test datasheet of water addition for soft material

10 Selection of sieve size for granulation: As the main purpose of granulation was to prevent the Lucidum spores being taken away by the extractant in case of supercritical fluid extraction. The sieve size was not important as long as the mixture can be prepared into particles, so when we selected the sieve size, we mainly investigated the molding rate of the particles through visual observation to control the ratio of fine powder. The particle molding rate of 10 mesh (stainless steel) was high
15 and the results were shown in Table 5.

Serial No.Stainless steel mesh	Particle situation
18	The ratio of fine powder was high.
210	The particle molding rate was about 90% with few fine powder.
312	There were noodle-shaped materials sticky on the sieve mesh.

Table 5: Test datasheet of sieve size

Therefore, the preferred granulation method was as follows: wall-broken Lucidum spores were added with distilled water in an amount of 25% by weight of the broken Lucidum spores, well mixed and prepared into soft material, and then the soft material was placed into a granulator followed by granulation with 10 mesh stainless steel sieve to obtain the Lucidum spores particles.

(2) Drying

Determination of drying temperature: the investigation of drying temperature mainly depended on the drying rate and the particle moisture. From the perspectives of production cost and ensuring the activity of Lucidum spores effective components, the drying temperature was determined as $\leq 60^{\circ}\text{C}$ and the drying time was 4 hours with moisture of $\leq 5\%$. The results were shown in Table 6.

Serial No.	Particle weight (kg)	Drying temperature ($^{\circ}\text{C}$)	Drying time (h)	Particle moisture (%)
1	2	40	6.5	5.2
2	2	60	4	5
3	2	80	3.5	4.9

Table 6: Test datasheet of drying temperature

d: the dry *Lucidum* spores particles obtained from the step c were mixed with *Atractylodes* rhizome obtained from the step a, and the mixture was carried out extraction of crude oil mixture and removal of impurities and moisture to give the mixture oil of *Lucidum* spores and *Atractylodes* rhizome.

- 5 (1) took dry *Lucidum* spores particles and mix with *Atractylodes* rhizome obtained from the step a;

(2) extracted the mixture oil of *Lucidum* spores and *Atractylodes* rhizome (crude oil mixture) through CO₂ super critical extraction.

Orthogonal test of process conditions: CO₂ supercritical fluid extraction technology is
 10 a relatively new extraction and separation technology, which uses the unique natures of extractant under the supercritical state to achieve the purpose of extraction and separation and has advantages of no chemical solvent residue, avoiding the pyrolysis of extractant under high temperature and ensuring the activity of physiologically active substances, etc. When extracting crude oil mixture of *Lucidum* spores and
 15 *Atractylodes* rhizome with CO₂ supercritical extraction technology, main factors include the particle size of extractive substances, extraction pressure, extraction temperature, extraction time, and CO₂ flow rate, and so on. On the basis of pretest, under the situation of fixed parameters with 1000g dry *Lucidum* spores particles and 10 mesh sieve, we used L₉ (3)⁴ orthogonal test on the main factors affecting the
 20 extraction efficiency, such as extraction pressure, extraction temperature, extraction time and CO₂ flow rate, and adopted the yield of crude *Lucidum* spores and *Atractylodes* rhizome oil mixture as indexes to carry out the test of extraction conditions. The results were shown in Table 7-8, Table 9-1 and Table 9-2.

Factors	A	B	C	D
Levels	Extraction pressure (MPa)	Extraction temperature (°C)	Extraction time (h)	CO ₂ flow rate (m ³ /h)
1	18	40	3	0.4
2	22	50	4	0.5
3	26	60	5	0.6

Table 7: Factor level of extraction technology

Serial No.	Factors	A	B	C	D	Oil quantity of crude Lucidum spores oil (g)
	Level	Extraction pressure (MPa)	Extraction temperature (°C)	Extraction time (h)	CO ₂ flow rate (m ³ /h)	
1		1	1	1	1	32.4
2		1	2	2	2	35.5
3		1	3	3	3	84.4
4		2	1	2	3	50.9
5		2	2	3	1	75.1
6		2	3	1	2	94.6
7		3	1	3	2	103.2
8		3	2	1	3	98.5
9		3	3	2	1	115.0

Table 9-1: Orthogonal table with Lucidum spores and Atractylodes rhizome oil mixture quantity as indexes

- 5 Note: There are nine test schemes. For example, No. 6 test was A₂B₃C₁D₂, namely extraction pressure was 22 MPa, extraction temperature was 60°C, extraction time was 3h, CO₂ flow rate was 0.5m³/h, and so on.

Parameters	A	B	C	D
	Extraction pressure	Extraction temperature	Extraction time	CO ₂ flow rate
	(MPa)	(°C)	(h)	(m ³ /h)
K1	152.300	186.500	225.500	222.500
K2	220.600	209.100	201.400	233.300
K3	316.700	294.000	262.700	233.800
K1	50.767	62.167	75.167	74.167
K2	73.533	69.700	67.133	77.767
K3	105.567	98.000	87.567	77.933
R	54.800	35.833	20.433	3.767

Table 9-2: Analytic results of orthogonal test data

With the quantity of crude *Lucidum* spores and *Atractylodes* rhizome oil mixture as indexes, the analysis results of range was $A > B > C > D$, namely the order of the influencing factors was extraction pressure > extraction temperature > extraction time > CO₂ flow rate, and the best combination of each factor and level was $A_3B_3C_3D_3$. D item was used as the error term to further carry out the variance analysis and the results were shown in Table 8.

Table Variance analysis with the quantity of crude *Lucidum* spores and *Atractylodes* rhizome oil mixture as indexes

Variance sources	Sum of deviation squares	Degree of freedom	Mean-square	F value	Significance
A	57386.180	2	28693.090	1.086	>0.05
B	54980.353	2	27490.177	1.040	>0.05
C	53474.500	2	26737.250	1.012	>0.05
Error	52865.860	2	26432.930		
Sum	165841.033	8			

- 10 The critical value result of F-test was $F_{0.05}(2,2) = 19.00$, and the influences of each factor ($P > 0.05$) on the experimental results were not significant.

Validation test of CO₂ supercritical fluid extraction process: in order to verify the results, the A₃B₃C₃D₃ conditions were selected to carry out the extraction test, to see whether the selected conditions were consistent with the original test results for validation. The results were shown in table 10.

5

Serial No.	A	B	C	D	Oil quantity of crude Lucidum spores oil (g)
	Extraction pressure (MPa)	Extraction temperature (°C)	Extraction time (h)	CO ₂ flow rate (m ³ /h)	
1	26	60	5	0.6	109.0
2	26	60	5	0.6	110.4
3	26	60	5	0.6	113.5

Table 10: Results of extraction validation test

Therefore, the yield of crude Lucidum spores and Atractylodes rhizome oil mixture in the validation test was consistent with the orthogonal results, and A₃B₃C₃D₃ was determined as the best extraction process conditions, namely extraction pressure of 26MPa, extraction temperature of 60°C, extraction time of 5h and CO₂ flow rate of 0.6m³/h.

(3) The obtained crude Lucidum spores and Atractylodes rhizome oil mixture was confirmed with plate-and frame type filtering or high-speed centrifugation, to eliminate the impurities and water to give bright and yellow Lucidum spores and Atractylodes rhizome oil mixture, namely the said Chinese medicine preparation.

The Lucidum spores and Atractylodes rhizome oil mixture can be directly filled into bottles for oral administration.

Embodiment 2 Preparation method for soft capsule of anti-tumor Chinese medicine

preparation containing *Lucidum* spores oil

The *Lucidum* spores and *Atractylodes* rhizome oil mixture was obtained according to the method in embodiment 1, gelatin, glycerin and water as raw materials were used to prepare soft capsule shell and then soft capsules were prepared through an encapsulating machine.

Embodiment 3 Preparation method for tablet of anti-tumor Chinese medicine preparation containing *Lucidum* spores oil

Clathrate compound of *Lucidum* spores and *Atractylodes* rhizome oil mixture (clathrate) was obtained by inclusion agent (β -cyclodextrin) according to the method in embodiment 1, and the preparation method was as follows:

(1) Laboratory instruments and reagents

TM-40 colloid mill, Tianjin Xinpu Machine Manufacture Co., Ltd.; LN-2102PC UV spectrophotometer, UNICO (Shanghai) Instruments Co., Ltd.; DZF-1B type vacuum drying oven, Shanghai Yuejin Medical Instrument Plant; CQX25-06 type ultrasonic cleaner, Branson Ultrasonics (Shanghai) Co., Ltd.; magnetic stirrer, Shanghai Sile Instrument Co., Ltd.; HH-S digital thermostat water bath, Changzhou National Test Equipment Institute; AB104-N type electronic analytical balance, Mettler-Toledo Instruments (Shanghai) Co., Ltd. β -cyclodextrin, Sinopharm Chemical Reagent Co., Ltd, batch number: F20060731; other reagents were of analytical grade.

(2) Selection of inclusion method

Compare the saturated aqueous solution method, ultrasonic method and grinding method under the same proportion of materials with clathrate yield, oil content and oil

utilization rate as indexes.

Saturated aqueous solution method: 16g of β -cyclodextrin was prepared into saturated solution under 60°C, and slowly added with 2ml of *Lucidum* spores and *Atractylodes* rhizome oil mixture at a constant temperature, stirred for a certain time, and stored at 4°C for 24 h followed by air pump filtration and washing with a small amount of water and ethanol for 3 times, drying at 40°C under vacuum to obtain β -cyclodextrin clathrate.

Ultrasonic method: 16g of β -cyclodextrin was added with 3 times amount of water, well mixed and slowly added with 2ml of *Lucidum* spores and *Atractylodes* rhizome oil mixture, and ultrasound for a certain time and other steps were the same as the above. ($f = 40$ KHz)

Grinding method: 16g of β -cyclodextrin was added with water whose amount is 3 times of β -cyclodextrin, well mixed and poured into a mortar, slowly add with 2ml of *Lucidum* spores and *Atractylodes* rhizome oil mixture, and ground for a certain time and other steps were the same as the above.

According to the experimental results (Table 11), grinding method was preferably used for β -cyclodextrin inclusion of *Lucidum* spores and *Atractylodes* rhizome oil mixture.

Methods	Yield/%	Oil utilization rate/ %	Oil content/%
Saturated aqueous solution method	70.6	65.78	13.67
Ultrasonic method	77.2	70.62	13.01
Grinding method	85.6	89.25	14.64

Table 11: Results comparison of three different inclusion methods

(3) Selection of inclusion conditions in grinding method

Orthogonal experimental design: It was known from literature and pre-experimental study that main factors of grinding method included four factors, such as material ratio of *Lucidum* spores and *Atractylodes* rhizome oil mixture to β -cyclodextrin (A), water addition (B), inclusion time (C), and alcohol consumption (D), so $L_9(3^4)$ orthogonal design table was used with clathrate yield, clathrate oil utilization rate and clathrate oil content as evaluation indexes, and the comprehensive scoring method was used to analyze and optimize the best technological conditions, in which the weight coefficients of clathrate yield, clathrate oil utilization rate and clathrate oil content were respectively 0.2, 0.4, and 0.4 (the factor level division was shown in Table 12, and the orthogonal experimental arrangement and results were shown in Table 13-1 and Table 13-2).

Factors	A	B	C	D
Level		(times)	(min)	(%)
1	1:4	2	20	10
2	1:6	3	30	20
3	1:8	4	40	30

Table 12: Factor level chart

Serial No.	A	B	C	D	clathrate yield (%)	Oil content (%)	Oil utilization rate (%)	Comprehensive scores
1	1	1	1	1	84.26	17.63	92.86	97.67
2	1	2	2	2	92.88	14.26	91.98	97.69
3	1	3	3	3	86.82	15.24	90.24	96.18
4	2	1	2	3	83.82	14.48	94.38	96.93
5	2	2	3	1	84.67	16.32	90.66	96.35
6	2	3	1	2	85.48	13.95	88.21	94.58
7	3	1	3	2	86.63	15.22	89.10	95.67
8	3	2	1	3	88.01	14.68	86.32	94.62
9	3	3	2	1	90.42	11.38	87.60	94.30

Table 13-1 Orthogonal experimental table for inclusion of *Lucidum* spores and *Atractylodes* rhizome oil mixture with β -cyclodextrin

Serial No.		A	B	C	D
Clathrate yield	K ₁	264.0	254.7	257.8	259.4
	K ₂	254.0	265.6	267.1	265.0
	K ₃	265.1	262.7	258.1	258.7
	R	3.7	3.6	3.1	1.9
Oil content	K ₁	47.13	47.33	46.26	45.33
	K ₂	44.75	45.26	40.12	43.43
	K ₃	41.28	40.57	46.78	44.40
	R	1.95	2.25	2.22	0.31
Oil utilization rate	K ₁	275.1	276.3	267.4	271.1
	K ₂	273.3	269.0	274.0	269.3
	K ₃	263.0	266.1	270.0	270.9
	R	4.02	3.43	2.19	0.61
Comprehensive scores	K ₁	291.5	290.3	286.9	288.3
	K ₂	287.9	288.7	288.9	287.9
	K ₃	284.6	285.1	288.2	287.7
	R	2.31	1.74	0.68	0.19

Table 13-2 Orthogonal experimental result analysis for inclusion of *Lucidum* spores and *Atractylodes* rhizome oil mixture with β -cyclodextrin.

Variance sources	Sum of deviation squares	Degree of freedom	Meansquare	F value	Significance p
A	82862.503	2	41431.25	1.00	>0.05
B	82859.216	2	41429.61	1.00	>0.05
C	82855.175	2	41427.59	1.00	>0.05
Error	82854.515	2	41427.26		
Sum	331431.408	8			

$$F_{0.05}(2, 2)=19.00$$

Table 14: Variance analysis table with comprehensive scores as indexes

After analyzed the results of clathrate comprehensive scores (table 14), it was shown from the range analysis that the influence of each factor to this index can be arranged as $A > B > C > D$ and there was no significant differences between each factor. Level of factor A from best to worst was $1 > 2 > 3$; level of factor B from best to worst was $1 > 2 > 3$; level of factor C from best to worst was $2 > 3 > 1$; level of factor D from best to worst was $1 > 2 > 3$, and the most preferable process with comprehensive scores as index was $A_1B_1C_2D_1$, namely the inclusion conditions were as follows: the weight ratio of *Lucidum* spores and *Atractylodes* rhizome oil mixture to β -cyclodextrin was 1: 4, the water addition amount was 2 times of the weight of *Lucidum* spores and *Atractylodes* rhizome oil mixture, the grinding time was 30 min and the anhydrous ethanol was 10% by weight of *Lucidum* spores and *Atractylodes* rhizome oil mixture.

Verification tests: three batches of validation tests were carried out according to the above most preferable process and the measurement results were shown in Table 15, which indicated that this process was stable and feasible.

Serial No.	Clathrate yield %	Oil content %	Oil utilization rate %
1	92.8	14.2	97.6
2	93.6	14.5	96.5
3	92.3	14.7	96.9
RSD	0.71	1.74	0.57

Table 15: Results of validation tests

(4) UV characteristics of clathrate

- 1g of *Lucidum* spores and *Atractylodes* rhizome oil mixture was taken and added with 20 mL of petroleum ether (30°C ~ 60°C), dissolved under ultrasonic and filtered, and the filtrate was stored as test solution 1; 0.15g of *Lucidum* spores and *Atractylodes* rhizome oil mixture was taken and added with 20 mL of petroleum ether (30°C ~ 60°C), dissolved under ultrasonic, added with 0.6g β -cyclodextrin, shaken and filtered, and the filtrate was stored as test solution 2; 1g of clathrate was taken and added with 20 mL of petroleum ether (30°C ~ 60°C), dissolved under ultrasonic and filtered, and the filtrate was stored as test solution 3; 1g of clathrate was taken and added with 20 mL of petroleum ether (30°C ~ 60°C), shaken and filtered, and the filtrate was stored as test solution 4; and 1g of β -cyclodextrin was taken and added with 20 mL of petroleum ether (30°C ~ 60°C), shaken and filtered, and the filtrate was stored as test solution 5.
- The UV spectrum of clathrate (Figure 2) showed that *Lucidum* spores and *Atractylodes* rhizome oil mixture, physical mixture of *Lucidum* spores and *Atractylodes* rhizome oil mixture and β -cyclodextrin, and substances after de-clathration had UV absorption, and the clathrate and β -cyclodextrin had no UV absorption, which indicated that new substances were formed after clathration of *Lucidum* spores and *Atractylodes* rhizome oil mixture and β -cyclodextrin, and the main component of *Lucidum* spores and *Atractylodes* rhizome oil mixture did not change after clathration.

Preparation of tablets: this clathrate was added with 10%-15% maltodextrin, well mixed and dry granulated, and then the resulting particles were added with 0.5% silica powder, well mixed and pressed into tablets (0.25g/tablet) followed by film-coating or sugar-coating to obtain tablets.

5 Embodiment 4 Preparation method for hard capsules of anti-tumor Chinese medicine preparation containing *Lucidum* spores oil

The clathrate was obtained according to the method in embodiment 3 and dry-granulated, and then the particles were filled into hard capsules (0.20g/tablet).

10 Embodiment 5 Study on primary pharmacodynamic effects of anti-tumor Chinese medicine preparation containing *Lucidum* spores oil - *Lucidum* spores and *Atractylodes* rhizome oil mixture and their clathrate

According to the requirements of National Guidance Principles of Pre-clinical study on Six Categories of New Drugs of TCM, the inhibition effects on mice-transplanted tumor sarcoma S₁₈₀ was investigated to verify the drug's main effects. Because the
15 main components of this Chinese medicine preparation was *Lucidum* spores and *Atractylodes* rhizome oil mixture, the effects of this oil mixture were verified; during the clathration process, the solubility of the *Lucidum* spores and *Atractylodes* rhizome oil mixture was significantly increased and the bioavailability of the essential oil was improved, and tablets and hard capsules were based on the clathrates, therefore, the
20 effects of various dosage forms can be validated through verifying the efficacy of the clathrate.

(1) Test materials

Tested drugs: clathrate of *Lucidum* spores and *Atractylodes* rhizome oil mixture, the

oil mixture was obtained according to the method in embodiment 1, batch No.: 20080101; pure *Lucidum* spores oil, batch No.: 20080112 (produced by Nanjing Zhongke Pharmaceutical Co., Ltd.); pure *Atractylodes* rhizome oil, batch No.: 20080101 (produced by Nanjing Zhongke Pharmaceutical Co., Ltd.); solvent: 0.5%
 5 CMC-Na; positive drugs (known anti-chemotherapy drugs): cyclophosphamide for injection (Shanghai Hualian Pharmaceutical Co., Ltd.), Specifications: 200mg / bottle, batch No.: 080304.

Animals: ICR mice, 18-22g, half male and half female, provided by Animal Room of China Pharmaceutical University, certification No.: SCXK(Jiangsu)2006-0011; C57
 10 mice, 18-22g, provided by Shanghai Animal Center of Chinese Academy of Science, certification No.: SCXK(Shanghai)2006-0010; feed: granulated feed, provided by Animal Room of China Pharmaceutical University; feeding conditions: air-conditioned room with temperature of 18-24°C and relative humidity of 70%.

Tumor strain Source: S₁₈₀ provided by Jiangsu Institute of Anti-tumor Materia
 15 Medica.

Instruments: YJ-875 type medical clean bench (Suzhou Purification Equipment Factory); TG-729B plastic electro-optical analytical balance (Shanghai Balance Instrument Factory); normal centrifuge (Beijing Medical Centrifuge Factory)

(2) Inhibition effects of various tested drug on mice-transplanted tumor solid
 20 sarcoma S₁₈₀

Experimental process: 60 above ICR mice were inoculated with S₁₈₀ solid sarcoma according to the research approach for transplanted tumors (tumor masses were obtained under sterile conditions, weighed and ground in a glass tissue homogenizer, and placed into sterile container after well ground, followed by adding normal saline
 25 to prepare into 1:3 of cell suspension. The container was placed on ice and pumped

with an empty syringe and cells should be well mixed before pump, and 0.2 ml was subcutaneously inoculated at the armpit of the right forelimb of each mouse), and mice were weighed 24h after inoculation and randomly divided into six groups: the control group (0.5% CMC-Na), the Lucidum spores and Atractylodes rhizome oil mixture group (1g/kg), the clathrate of Lucidum spores and Atractylodes rhizome oil mixture group (1g/kg), pure Lucidum spores oil group (1g/kg), pure Atractylodes rhizome oil group (1g/kg), and cyclophosphamide group (20mg/kg), and these mice were administrated 24h after inoculation through ig, once a day for 7 days. The tumor-bearing mice were killed at 2 days after drug withdrawal and weighed, and the tumor masses were seperated and weighed and the obtained data were statistically analyzed (t test).

The experimental results were as follows: compared with S₁₈₀ control group, other tested drugs had significant inhibition effects on S₁₈₀ tumor growth ($P<0.01$, $P<0.05$). The tumor inhibitory effects in the Lucidum spores and Atractylodes rhizome oil mixture group (1g/kg) and the clathrate of Lucidum spores and Atractylodes rhizome oil mixture group (1g/kg) were significantly higher than that in pure Lucidum spores oil group (1g/kg) and pure Atractylodes rhizome oil group (1g/kg); in which the inhibitory effect in the clathrate of Lucidum spores and Atractylodes rhizome oil mixture group (1g/kg) was most prominent. The Lucidum spores and Atractylodes rhizome oil mixture, the clathrate of Lucidum spores and Atractylodes rhizome oil mixture, pure Lucidum spores oil and pure Atractylodes rhizome oil had no significant impact on the growth of mice body weight ($P>0.05$), and cyclophosphamide had significant inhibitory effect on the growth of mice body weight ($P<0.05$). The experiment was repeated for three times, and the results were similar (Table 16).

Batches	Groups	Dosage (mg/kg)	Body weight (g)		Tumor weight (g)	Tumor inhibitory rate (%)
			Before administration	After administration		
1	CMC		19.68±1.29	26.11±2.73	1.72±0.41	0.00
	Lucidum spores and Atractylodes rhizome oil mixture	1000	19.87±1.25	27.30±2.38	0.92±0.32	46.01**
	clathrate of Lucidum spores and Atractylodes rhizome oil mixture	1000	19.98±1.18	26.36±2.40	0.81±0.29	52.11**
	pure Lucidum spores oil	1000	19.71±1.18	26.91±1.91	1.10±0.22	35.96**
	pure Atractylodes rhizome oil	1000	19.70±1.19	26.35±2.01	1.17±0.20	31.98**
	CY	1000	19.31±1.10	23.61±1.72*	0.61±0.20	64.34**
2	CMC		20.07±1.25	26.12±2.44	1.87±0.46	0.00
	Lucidum spores and Atractylodes rhizome oil mixture	1000	20.03±1.22	26.35±2.00	1.02±0.31	45.45**
	clathrate of Lucidum spores and Atractylodes rhizome oil mixture	1000	20.04±1.21	26.60±2.07	0.90±0.29	51.87**
	pure Lucidum spores oil	1000	20.34±1.12	26.70±2.15	1.21±0.42	35.63**
	pure Atractylodes rhizome oil	1000	20.35±1.17	26.39±2.37	1.29±0.38	31.02**
	CY	1000	20.27±1.52	24.10±1.44*	0.73±0.24	61.22**
3	CMC		19.91±1.23	26.48±2.30	1.66±0.39	0.00
	Lucidum spores and Atractylodes rhizome oil mixture	1000	19.96±1.24	27.07±1.62	0.89±0.23	46.38**
	clathrate of Lucidum spores and Atractylodes	1000	19.93±1.30	26.38±1.86	0.79±0.77	52.40**

	rhizome oil mixture					
	pure Lucidum spores oil	1000	20.00±1.16	27.02±2.04	1.04±0.40	37.46**
	pure Atractylodes rhizome oil	1000	20.02±1.09	28.31±2.35	1.11±0.32	33.13**
	CY	20	19.83±1.22	24.46±1.68*	0.65±0.17	60.79**

Table 16: Influences of each tested drug on the mice-transplanted tumor solid sarcoma
S₁₈₀

In which, the body weight and the tumor weight were indicated as ($\bar{X} \pm SD$) and the number of samples in each group was 10. "*" indicated that $P < 0.05$ in the corresponding data and "***" indicated that $P < 0.01$ in the corresponding data, compared with the control group.

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CLAIMS

1. An anti-tumor Chinese medicine preparation containing Lucidum spores oil, which is characterized in that the raw materials of the Chinese medicine preparation are Lucidum spores and Atractylodes rhizome.
- 5 2. The Chinese medicine preparation according to claim 1, which is characterized in that the ratio of Lucidum spores and Atractylodes rhizome by weight is 1:1.
3. The Chinese medicine preparation according to claim 1 or 2, which is characterized in that the above mentioned raw materials are combined with
10 pharmaceutically acceptable excipient to prepare into various pharmaceutical oral dosage forms.
4. A method for preparing the Chinese medicine preparation according to claim 1 or 2, the specific steps of which are as follows:
 - a: respectively clean Lucidum spores and Atractylodes rhizome, and respectively
15 weigh the cleaned Lucidum spores and Atractylodes rhizome according to the weight ratio;
 - b: break the Lucidum spores obtained from the step a through wall-breaking and grinding;
 - c: broken Lucidum spores obtained from the step b are granulated and dried to
20 give dry Lucidum spores particles;
 - d: the dry Lucidum spores particles obtained from the step c are mixed with

Atractylodes rhizome obtained from the step a, and the mixture is carried out extraction of crude oil mixture and removal of impurities and moisture to give the mixture oil of Lucidum spores and Atractylodes rhizome, namely the above mentioned Chinese medicine preparation.

5 5. The preparation method according to claim 4, which is characterized in that the method for extracting the crude oil mixture is CO₂ super critical extraction.

6. The preparation method according to claim 5, which is characterized in that the conditions of CO₂ super critical extraction for extracting crude oil mixture are as follows: the extraction temperature is 40 to 60°C, the pressure is 18 to 26 MPa,
10 CO₂ flow rate is 0.4 to 0.6m³/h, and the extraction time is 3 to 5 h.

7. The preparation method according to claim 6, which is characterized in that the Lucidum spores oil mixture is prepared into soft capsules through conventional methods.

8. The preparation method according to claim 6, which is characterized in that
15 the Lucidum spores and Atractylodes rhizome oil mixture was included with β -cyclodextrin to obtain the clathrate of the the Lucidum spores and Atractylodes rhizome oil mixture.

9. The preparation method according to claim 8, which is characterized in that the clathration method was as follows: 4~8 parts by weight of β -cyclodextrin and
20 2~4 parts by weight of water are well mixed, and added with 1 part by weight of Lucidum spores and Atractylodes rhizome oil mixture, ground for 20~40 min and stored under low temperature followed by air pump, washing with 0.1 to 0.3 parts by weight of anhydrous ethanol and drying under vacuum to obtain the clathrate of the Lucidum spores and Atractylodes rhizome oil mixture.

10. The preparation method according to claim 6, which is characterized in that the clathrate is prepared into tablets or hard capsules.

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