Preparation of \textit{Radix Arnebiae} ointment

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Abstract: Aim of this study is to prepare Arnebia ointment research, screening, get the best preparation technology. The preparation process of different frying temperature, different frying time, different oil quantity and dosage ratio were prepared by frying method. The orthogonal method was used in this study. The content of L-shikonin in Arnebiae ointment was used as the research factor, and the preparation process of Arnebiae ointment was optimized by this method. The experimental results show that the optimum extraction process is the extraction temperature of 110°C, the holding time is 1.5min, and when the ratio of oil to oil is 1:8, the quality of Arnebiae ointment the best. The extraction process can effectively improve the L-shikonin in the Arnebiae Ointment, and the extraction process is reasonable, efficient and economical.

1. Introduction
Arnebiae is a plant belonging to the genus Arnebiae\textsuperscript{[1]}. Arnebiae has the function of resisting bacteria, inflammation, viruses and tumors. It can regulate immunity, protect liver, resist oxidation, invigorate blood circulation, detoxify and promote eruption\textsuperscript{[2]}. It is reported in the literature that the activity of shikonin ointment against bacteria and inflammation is very strong\textsuperscript{[3]}. This article adopts the frying method, using the method of ultraviolet spectrophotometry\textsuperscript{[4-6]} to determine the content of the preparation of levo shikonin of Arnebia euchroma oil under different technological conditions, to study the different preparation process on the content of L-shikonin, finally to determine the reasonable extraction process. In this paper, orthogonal experimental\textsuperscript{[8-10]} design was used to select the best preparation process of Arnebiae ointment, The content of L-shikonin was the index.

2. Experimental apparatus and medicinal materials

2.1 Experimental material
Arnebiae (Harbin Shunhe pharmacy); the leaves of Ilex chinensis. (Harbin Shunhe pharmacy); Borneol (Harbin Shunhe pharmacy); the reference substance of L-shikonin (China Institute about Pharmaceutical and Biological Products)

2.2 Experimental instrument
Digital electric thermostatic water bath (Jintan City, Jiangsu Province, Rong Hua Instrument Manufacturing Co Ltd); Type 722 UV visible spectrophotometer (Shanghai Chaojing science and Technology Co. Ltd.); METTLER TOLEDO AG135 electronic balance
3. Experimental part

3.1 Preparation process

3.1.1 Experimental design of orthogonal method
The pilot will find the main factors affecting the quality of Arnebiae ointment preparation temperature (A), frying time (B), the charge quantity and the amount of oil ratio (C) of three, adopted a gradient experiment design, orthogonal test design is shown in table 1.

| Factor   | Level | A (Temperature/°C) | B (Time/min) | C (Dose to oil ratio) |
|----------|-------|-------------------|--------------|----------------------|
| 1        | 80    | 1.0               |              | 1:8                  |
| 2        | 110   | 1.5               |              | 1:10                 |
| 3        | 140   | 2.0               |              | 1:12                 |

3.1.2 Experimental method
The preparation of Arnebiae ointment is mainly made up of Arnebiae 40g, leaves of Ilex chinensis 10g, borneol 4g, beeswax 4g, and a certain amount of vegetable oil. After frying Arnebiae for a period of time, add leaves of lex chinensis. fried refining, with gauze filter when it is very hot, the filter residue is removed, and the temperature dropped to 60 degrees when adding borneol stirring.

3.2 L-SHIKONIN Determination content

3.2.1 Preparation of sample solutions
The amount of ethanol added to each Arnebiae ointment for dissolved 10mL volume to get the sample flask.

3.2.2 Method for selecting maximum absorption wavelength
The L-shikonin reference substance solution was placed on an ultraviolet spectrophotometer for wavelength scanning. It was found that the maximum absorbance of the Arnebiae reference solution was at a wavelength equal to 516 nm, so the wavelength of the quantitative detection was selected at 516 nm.

3.2.3 The method of determination
AL-shikonin accurately weighed 5g Arnebiae Ointment into the evaporating dish in the water bath method evaporated, and then dissolved in 30mL of ethanol extract, weighed and shaken after weighing, if the weight loss part of the use of ethanol to fill and then filter will The filtrate was dissolved in ethanol into a 50 mL volumetric flask. The absorbance was measured by ultraviolet spectroscopy at a wavelength of 516 nm.

3.3 The L-shikonin of the methodology

3.3.1 A Study of Linear Relationship
With the pipette, the amount of L-shikonin reference solution 0mL, 1mL, 2mL, 3mL, 4mL, 5mL, 6mL were placed in 10 mL volumetric flask, in the flask with 60% ethanol volume to 5.0mL. After standing for 1 mL, the absorbance was measured at a wavelength of 516 nm. The regression equation is: \( Y = 12.02x - 0.0047, R^2 = 0.9998 \), and the equation shows that the abscissa is the standard curve, where the ordinate is the absorbance value A and the abscissa is the L-shikonin concentration C (mg / mL) Method linear relationship is good, can be used as L-shikonin determination method.
Table 2. The data of L-shikonin standard curve

| Concentration C(mg/mL) | Absorbance (A) |
|------------------------|----------------|
| 0.00 0.01 0.02 0.03 0.04 0.05 | 0.000 0.110 0.235 0.355 0.479 0.596 |

3.3.2 Precision experiment
The relative standard deviation (RSD) was 0.18% <2%, which indicated that the method was L-shikonin urate and L-shikonin urate, and the determination condition was easy to grasp. It was proved that this method could be used.

Table 3. The determination of L-shikonin method of precision

| Sample number | Absorbance (A) | Absorbance of the average | RSD (%) |
|---------------|----------------|----------------------------|---------|
| 1             | 0.396          |                            |         |
| 2             | 0.396          |                            |         |
| 3             | 0.397          | 0.396                      | 0.18%   |
| 4             | 0.396          |                            |         |
| 5             | 0.395          |                            |         |
| 6             | 0.395          |                            |         |

3.3.3 Stability experiment
The results showed that the content of L-shikonin was stable at 0h, 1h, 4h, 8h, 12h and 24h respectively. The results showed that the content of L-shikonin was stable at the wavelength of 516nm. The relative standard deviation was 0.60% Because it is less than 2%, indicating that the spectrophotometric determination of L-shikonin content is stable and reliable.

Table 4. The results of the stability test

| Time (h) | Absorbance (A) | Absorbance of the average (A) | RSD (%) |
|---------|----------------|-------------------------------|---------|
| 0       | 0.689          |                               |         |
| 1       | 0.687          |                               |         |
| 2       | 0.690          |                               |         |
| 4       | 0.691          | 0.692                         | 0.60%   |
| 8       | 0.697          |                               |         |
| 12      | 0.696          |                               |         |
| 24      | 0.697          |                               |         |

3.3.4 Repetitive experiment
L-shikonin ording to the preparation method, 6 parts of ARNEDIAE OINTMENT were prepared. The absorbance was measured 6 times in parallel and the content of L-shikonin was calculated. The average absorbance was 0.690 and the relative standard deviation was 0.62%. It was less than 2%, which indicated that the repeatability was good.
Table 5. The experiment was conducted to calculate the recovery of the sample

| Sample Number | Absorbance (A) | Absorbance of the average (A) | RSD (%) |
|---------------|----------------|-------------------------------|---------|
| 1             | 0.673          |                               |         |
| 2             | 0.700          |                               |         |
| 3             | 0.701          | 0.690                         | 0.62%   |
| 4             | 0.689          |                               |         |
| 5             | 0.696          |                               |         |
| 6             | 0.681          |                               |         |

3.3.5 The recovery rate of the experiment

The average recoveries were 101.34 and the relative standard deviations were 1.91%, less than 2% within the allowable range, and the average recoveries were determined by the addition of 1 mL L-shikonin of the control stock solution. It can be determined that the sample recovery rate is relatively good.

Table 6. The test of sample recovery

| Sample Number | The content of the sample (mg) | The content of the reference substance (mg) | Measured content (mg) | Recovery rate (%) | The average of the recovery (%) | RSD (%) |
|---------------|--------------------------------|---------------------------------------------|-----------------------|------------------|--------------------------------|---------|
| 1             | 2.50                           | 5.50                                        | 7.98                  | 99.10            | 101.34                         | 1.91    |
| 2             | 5.00                           | 5.50                                        | 10.52                 | 100.50           |                                |         |
| 3             | 7.50                           | 5.50                                        | 13.26                 | 103.50           |                                |         |
| 4             | 1.00                           | 5.50                                        | 15.65                 | 101.50           | 101.34                         | 1.91    |
| 5             | 12.50                          | 5.50                                        | 18.46                 | 103.70           |                                |         |
| 6             | 15.00                          | 5.50                                        | 20.45                 | 99.70            |                                |         |

3.4 The results of the experiment

3.4.1 The results of the orthogonal test protocol

Table 7. The results of the orthogonal test protocol

| No. | A | B | C | L-SHIKONIN |
|-----|---|---|---|------------|
|     | 1 | 1 | 1 | 2.7706     |
| 2   | 1 | 1 | 2 | 2.9462     |
| 3   | 1 | 3 | 3 | 4.1265     |
| 4   | 2 | 1 | 2 | 5.7413     |
| 5   | 2 | 3 | 3 | 3.5468     |
| 6   | 2 | 3 | 1 | 5.3720     |
| 7   | 3 | 1 | 3 | 3.2158     |
| 8   | 3 | 3 | 2 | 4.4796     |
| 9   | 3 | 3 | 2 | 2.4165     |
| K1  | 9.8433 | 11.7277 | 12.6222 |
| K2  | 14.6601 | 10.9726 | 11.1040 |
| K3  | 10.1119 | 11.9150 | 10.8891 |
| R   | 4.8168 | 0.9424 | 1.7331 |
3.4.2 Analyze the results
The results show, RA>RC>RB, Indicating that the temperature is the greatest impact on the quality of the preparation of Arnebiae Ointment, the second factor is the ratio of herbs and liquid, the smallest factor is the Arnebiae holding time, so analyze the time as an error term. The results are below.

Table 8. the comprehensive analysis of variance analysis

| The source of variance | SS    | V | MS     | F      | P value |
|------------------------|-------|---|--------|--------|---------|
| A                      | 0.3211| 2 | 0.1605 | 13.65  | <0.05   |
| B                      | 0.0267| 2 | 0.0133 |        |         |
| C                      | 0.0575| 2 | 0.0287 | 0.47   | <0.05   |

The results of analysis of variance show that the temperature and the ratio of liquid to liquid are the key factors, which have a significant effect on the yield of L-shikonin. In summary, Arnebiae Ointment is the best preparation process temperature 110 ℃, holding time 1.5min, dose and oil ratio is 1:8.

4. Discussion
Arnebiae Ointment can spill blood, scattered bruises, toxins, remove rot, generate new texture, pain and other effects. Arnebiae Ointment has a strong anti-infective and anti-acute inflammation. Arnebiae Ointment is a topical ointment, so it has a very good biofilm shielding effect, there is a strong anti-infective effect. Arnebiae Ointment NT in clinical use for the treatment of skin burns, burns and dry skin, chapped and other symptoms.

Acknowledgments
This work was supported by Harbin municipal science and technology bureau project(2016RQQXJ124, 2016RAXXJ064); Innovation talent project of education department of heilongjiang province(UNPYSCT-2016181); Harbin University of Commerce graduate student innovation project(YJSCX2017-455HSD)

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