Optimization of Extraction Process of Valeriana officinalis L. Root Essential Oil and Study on Its Anti-Free Radical Activity

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Abstract. Taking Valerianrhizome as raw material and the yield of volatile oil of Valerian as an index, the extraction process of valerian volatile oil was analyzed by microwave assisted steam distillation, and optimized by Box-Behnken response surface test. The effects of scavenging capacity of Li, DPPH and hydroxyl radicals on their antioxidant capacity were studied. The results showed that the optimal process conditions of microwave extraction of volatile oil of Valerian were 350W microwave power, extraction time 25min, liquid-to-material ratio 8.8mL:1.0g, and the yield of volatile oil of Valerian extracted under this condition was 5.88%. Microwave-extracted volatile oil of Valerian has strong scavenging ability and high reducing power to DPPH free radicals and hydroxyl free radicals. Compared with steam distillation, it has obvious differences, which can provide reference for the development of antioxidant products of Valerian oil.

1. Introduction
Valeriana officinalis L. is Valerianaceae Valerian, a perennial herb with pharmacological activity that regulates the circulatory system and anti-oxidation and anti-tumour[1]. Valerian contains volatile oil, flavonoid, iridoids, alkaloids and other active ingredient, of which volatile oil is the main active ingredient of Valerian. At present, the extraction method of volatil oil of Valerian is mainly steam distillation, but it has high energy consumption, time consumption, low extraction efficiency, and poor appearance and smell of essential oil[2]. The method of super-critical fluid extraction of essential oil is efficient, high oil yield, safe and environmentally friendly, but the cost is relatively high[3]. At present, microwave-assisted extraction technology has been widely used in the extraction of essential oils. This method can complete the extraction in a short time, reducing the pyrolysis, hydrolysis, oxidation, etc. Of oxygenated compounds caused by the high temperature of the essential oil for a long time[4]. It has high energy utilization and extraction. It has the characteristics of fast rate, high yield, and can maintain the natural quality of volatile oil [5]. In this study, the extraction rate of Valerian essential oil was used as the main indicator. Through single-factor and Box-Behnken response surface test, the microwave-assisted steam distillation extraction process conditions were optimized to determine the reducing power, DPPH scavenging power, and hydroxyl radical scavenging power[6]. The antioxidant capacity of the volatile oil obtained by distillation is compared in order to provide a basis for the research on the medicinal and edible products of Valerian.
2. Materials and methods

2.1. Test materials
The roots of Valerian are collected from the Valerian planting base of Jiamusi University Science Park. Hexane, absolute ethanol, potassium ferricyanide, Tianjin Reagent Co., Ltd.; all other reagents used are of analytical grade. Scientz-DM microwave light wave ultrasonic extraction instrument, METTLER BIOTECH CO., LTD.; MA1000B electronic balance, Sartorius Scientific Instrument Co., Ltd.; rotary evaporator, Shanghai Shangtian Precision Instrument Co., Ltd.; UV-2550 type ultraviolet-visible spectrophotometer Meter, METTLER TOLEDO International Co., Ltd.

2.2. Test method

2.2.1. Extraction process of volatile oil of Valerian. Weigh an appropriate amount of sample, add NaCl solution, shake and mix and soak. Connect the essential oil tester to the re-flux condenser. After distillation for a period of time, let stand, take the essential oil, add anhydrous sodium sulfate, and filter to obtain the Valerian essential oil extract. Compare with the microwave-assisted Valerian essential oil for antioxidant activity. Microwave assisted extraction of essential oils for extraction. Take an appropriate amount of Valerian, add n-hexane to the round bottom flask, and place it in a microwave oven. Set the microwave power and time, cool to room temperature after re-flux extraction, filter with suction, collect the filtrate, concentrate in an appropriate amount, and evaporate in a water bath to obtain a microwave extract. The formula for calculating the yield of essential oils is as follows:

\[
Y = \frac{W_{V. officinalis oil (g)}}{W_{V. officinalis root (g)}} \times 100 \%
\]

(1)

2.2.2. Single factor investigation of microwave-assisted extraction process. This test is aimed at three factors: microwave power (200, 300, 400W), extraction time (10, 25, 30min), liquid-to-material ratio (4mL: 1g, 6mL: 1g, 8mL: 1g, 10mL: 1g, 12mL: 1g). Keeping two of the variables fixed and unchanged, a single factor test was conducted on the other variable, and the essential oil extraction rate was used as an indicator to determine the horizontal range required by the Box-Behnken response surface design.

Tab. 1 BO-Behnken response test factors and levels

| Level | Microwave power (W) | Extraction time(min) | Liquid-to-material ratio (mL/g) | Temperatu re | Accessories |
|-------|---------------------|----------------------|-------------------------------|-------------|-------------|
| -1    | 200                 | 15.0                 | 6:1                           | 20℃         | 2 %         |
| 0     | 300                 | 25.0                 | 10:1                          | 30℃         | 2 %         |
| 1     | 400                 | 30.0                 | 15:1                          | 38℃         | 2 %         |

2.2.3. Box-Behnken method Valerian essential oil extraction process. Based on the single-factor test, the microwave power, extraction time, and liquid-to-material ratio were selected as three factors that had a significant influence on the extraction rate of essential oils. The Box-Behnken test provided by Design-Expert 8.0.6 was used to optimize the extraction rate of essential oils. Extract the process parameters, and the experimental design (in Tab 1 and Tab 2).
### 2.2.4. Determination of DPPH free radical scavenging ability.

Ethanol as solvent, the concentration of essential oils formulated 0.1, 0.2, 0.3, 0.4, 0.5mg/mL solution for use. 300μL solution to learn the different concentrations, with 2mL 0.004% DPPH solution was mixed at room temperature after standing 20min, measured absorbance D517nm (1) at at 517nm. To extract without DPPH blank control, positive control of vitamin C, the absorbance was measured at 517nm D517nm (0). 300μL accurately learn different concentrations above oil solution, were mixed and ethanol, with ethanol as a control, and measured the absorbance of each solution 2mL anhydrous at 517nm  D517nm (2). Measuring the extracted oil by steam distillation of DPPH radical scavenging capacity by the same method, is calculated as follows:

\[
C_{DPPH} = \frac{1-(D_{517\text{nm}(1)} - D_{517\text{nm}(2)})}{D_{517\text{nm}(0)}} \times 100\% \tag{2}
\]

### 2.2.5. Determination of hydroxyl radical scavenging rate of valerian essential oil.

Prepare essential oil sample solution, take 1.5mL sample, add 1.0mL 2.5mmol/L salicylic acid solution, 1.0mL 5mmol/L FeSO₄ solution and 2.0mL distilled water, mix well, add 1.0mL 5mmol/L H₂O₂ at 510nm The absorbance was measured, distilled water was used as a blank reference, and vitamin C was used as a positive control. The hydroxyl radical scavenging rate of valerian essential oil extracted by steam distillation is determined as the formula:

\[
C_{\text{Hydroxyl radical}} = \left(1- \frac{D_{510\text{nm}(0)}}{D_{510\text{nm}(2)}} \right) \times 100\% \tag{3}
\]

### 3. Results and analysis

#### 3.1. Single factor investigation of microwave-assisted extraction process of oils

**3.1.1. The effect of microwave power on the extraction rate of essential oils.** With the increase of microwave power, the extraction rate of essential oils showed a trend of increasing first and then decreasing. When the microwave power is 300W, the extraction rate of essential oil is 5.88%. The reason for this is mainly due to the fact that when the power is increased, the temperature is too high, and the low-boiling substances are lost as the medium boils. Therefore, 150 – 450W is selected as the optimal level of response surface design(in Fig 1).
3.1.2. Effect of extraction time on the extraction rate of essential oils. As the extraction time increases, the extraction rate of essential oils tends to rise first, then stabilize at a certain level and then decrease. When the extraction time is 20-30min, the extraction rate is stable at about 4.88%. When the extraction time continues to increase, the essential oil is destroyed or volatilized from the medium and the extraction rate decreases. Therefore, 15 - 30min is selected as the extraction time range of response surface design (in Fig 2).

3.2. Box-Behnken response surface design optimization
Microwave power, extraction time, liquid-to-material ratio have extremely significant effects on the extraction rate of essential oils in the second term (P <0.01), and the influence among other factors is not significant. The results of Table 3 are in agreement with the interaction between the various factors reflected in the response surface diagram in Fig.4. According to Design-Expert 8.0.6 software, the optimal process parameters for essential oil extraction are microwave power 326.30W, extraction time 25.48min, liquid-material ratio 8.57mL: 1g, and the extraction rate of essential oil under this condition is 5.89%. In order to facilitate the production test requirements, the microwave power was set to 330W, the extraction time was 25min, the liquid-to-material ratio was 8.6mL: 1g, and further verification tests were conducted, and the extraction rate of essential oil was (5.89 ± 0.05)%. This value is relatively close to the predicted value, so microwave power 340W, extraction time 25min, liquid-to-material ratio 8.6mL: 1.0g are selected as the microwave-assisted extraction of Valerian essential oil process parameters (in Tab 3).
3.3. Antioxidant activity of Valerian essential oil in different processes

3.3.1. Determination of DPPH free radical scavenging ability. Valerian essential oils obtained by steam distillation and microwave-assisted extraction have a strong ability to scavenge DPPH free radicals, and there is a positive correlation between the concentration of Valerian essential oil and DPPH scavenging rate. When the concentration of Valerian essential oil was 0.5 mg / mL, the microwave-assisted extraction method had a clearance rate of 80.9%, while the steam distillation method had a clearance rate of 70.2% (in Fig 4).

| Source of variance | Sum of square | Degrees of freedom | Mean square | F value | P value |
|--------------------|--------------|--------------------|-------------|---------|---------|
| Regression model   | 14.91        | 9                  | 1.66        | 63.97   | <0.0001 |
| A                  | 2.98         | 1                  | 2.98        | 114.93  | <0.0001 |
| B                  | 0.33         | 1                  | 0.33        | 12.82   | 0.0090  |
| C                  | 0.003612     | 1                  | 0.003612    | 0.14    | 0.7199  |
| AB                 | 0.006400     | 1                  | 0.006400    | 0.25    | 0.6344  |
| AC                 | 0.020        | 1                  | 0.020       | 0.76    | 0.4132  |
| BC                 | 0.39         | 1                  | 0.39        | 15.08   | 0.0060  |
| A2                 | 9.83         | 1                  | 9.83        | 379.43  | <0.0001 |
| B2                 | 0.32         | 1                  | 0.32        | 12.32   | 0.0099  |
| C2                 | 0.49         | 1                  | 0.49        | 18.82   | 0.0034  |
| Residual           | 0.18         | 7                  | 0.026       |         |         |
| Mismatch           | 0.15         | 3                  | 0.049       | 5.80    | 0.0612  |
| Net error          | 0.034        | 4                  | 0.00847     | --      | --      |
| sum                | 15.09        | 16                 | --          | --      | --      |

Valerian essential oils obtained by steam distillation and microwave-assisted extraction have a strong ability to scavenge DPPH free radicals, and there is a positive correlation between the concentration of Valerian essential oil and DPPH scavenging rate. When the concentration of Valerian essential oil was 0.5 mg / mL, the microwave-assisted extraction method had a clearance rate of 80.9%, while the steam distillation method had a clearance rate of 70.2% (in Fig 4).

3.3.2. Determination results of essential oil reducing power. The Valerian essential oils obtained by steam distillation and microwave-assisted extraction have strong reducing power, indicating that the Valerian essential oils also have strong antioxidant capacity. In the measured mass concentration range, the reducing power of Valerian essential oil increases with the increase of concentration, and the reducing power of microwave-assisted extraction of Valerian essential oil is significantly higher than that of general steam distillation. When the concentration of Valerian essential oil is 0.5mg / mL, the absorbance of microwave-assisted extraction is (in Fig 6).
4. Conclusions
Response surface method has been widely used in various fields such as chemical industry, food, agriculture, forestry, metallurgy. It has the advantages of less test times and simple design. In this study, Valerian was used as the main raw material, and Box-Behnken response surface design was used to optimize the microwave-assisted extraction process conditions of essential oils, and the antioxidant properties of microwave-assisted extraction of essential oils and those obtained by steam distillation were studied. The results show that the optimal process conditions for microwave-assisted extraction of essential oils are microwave power 330w, extraction time 25min, liquid-to-material ratio 8.6ml: 1g, and the yield of Valerian essential oil extracted under this condition is 5.88%. The extraction process of essential oil in this experiment is stable and feasible, with high extraction rate and strong reducing ability. It has strong scavenging ability to DPPH free radicals and hydroxyl free radicals, and its antioxidant capacity is significantly different from the general steam distillation method. Microwave-assisted extraction is a new technology of energy-saving, high-efficiency, extraction of high-quality essential oils from plants, which has a good application prospect in plant development and food research.

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