Optimization of Flash Extraction Process and Antioxidant Activity in Vitro of Ligustrum Lucidum Seed Oil

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Abstract. Taking the extraction voltage, liquid-solid ratio and extraction time as the factors, and the yield of Ligustrum lucidum seed oil as the evaluation index, response surface methodology was used to explore the best technology of Ligustrum lucidum seed oil flash extraction method, and its antioxidant activity was analyzed. The results showed that the optimum extraction conditions were as follows: voltage 140 V, ratio of liquid to material 12:1, extraction time 150s, and the oil yield was 13.53%. The results showed that Ligustrum lucidum seed oil had certain scavenging ability to DPPH · free radical and hydroxyl radical, and its scavenging ability were positively correlated with its mass concentration. When the mass concentration was 2.5mg/ml, the DPPH · free radical scavenging rate was 32.25% and the hydroxyl radical scavenging rate was 43.83%.

1. Introduction
Ligustrum lucidum seed is the dry and mature fruit of Oleaceae plants *Ligustrum lucidum* Ait., which has the functions such as nourishing Yin Qi, calming liver fire, strengthening muscles and bones, nourishing liver and kidney, and black hair. It is sweet, bitter and cool in nature. It enters the liver and kidney channels. It contains flavonoids, terpenoids, polysaccharides, volatile oil, fatty oil and other active components [1-2]. It is reported that the content of fatty oil in Ligustrum lucidum seed can reach 13.2%, and the content of unsaturated fatty acid is as high as 87% [3-4]. It is found that Ligustrum lucidum seed oil has good antioxidant and anti-aging effects, and has high medical and health value [5]. At present, there are few studies on the extraction of Ligustrum lucidum seed oil. Only Lin Yihe and others use Soxhlet extraction method [4], Bian Yongling et al [6], compared with the traditional extraction method and ultrasonic extraction method, Soxhlet extraction and ultrasonic extraction have the advantages of simple equipment and convenient operation compared with traditional pressing method and supercritical CO2 extraction method. However, compared with modern technology, flash extraction still has the disadvantages of large solvent consumption, slow extraction speed, low efficiency and high energy consumption [7]. Flash extraction is a new type of oil extraction technology, which relies on high-speed mechanical shear force and super power molecular penetration technology, which can break plant materials into fine particles within seconds, so that the solvent can quickly penetrate into the plant tissue and realize rapid dissolution and extraction [8-11]. Therefore, it has the advantages of short extraction time, high efficiency and low solvent consumption, and its application prospect is very broad.
In this study, the flash extraction method was used to extract the oil from Ligustrum lucidum seed. The oil yield was taken as the evaluation index. The extraction process was optimized by response surface methodology, and the antioxidant activity of the oil in vitro was analyzed, so as to lay the foundation for its further comprehensive development and utilization.

2. Instruments and materials

2.1 Instrument
LN22-JHBE50T flash extractor (Beijing Zhongxi Yuanda Technology Co., Ltd.); RE-52A rotary evaporator (Shanghai Yarong biochemical instrument factory); QJ-04 Qijian brand high speed multifunctional pulverizer (Shanghai zhaoshen Technology Co., Ltd.); FA2004N electronic analytical balance (Shanghai Jinghai Instrument Co., Ltd.); UV-1800 ultraviolet visible spectrophotometer (Shanghai Macy Instrument Co., Ltd.); TG16-WS high speed frozen centrifuge (Changsha Xiangrui centrifuge Co., Ltd.).

2.2 Aganitia and reagents
Sample: Ligustrum Lucidum Ait. Was purchased from Bozhou traditional Chinese medicine market. It was identified as the dried and mature fruit of Ligustrum lucidum Ait. by Chen Na, associate professor of school of pharmacy, Bozhou Vocational and Technical College.
Reagents: petroleum ether (60 ~ 90 ℃), ascorbic acid (VC), anhydrous sodium sulfate, disodium hydrogen phosphate, trichloroacetic acid, ammonium persulfate, hydrogen peroxide, etc. are all analytical pure, purchased from Jiangsu Qiangsheng Functional Chemical Co., Ltd.; 1,1_diphenyl_2-picrylhydrazyl (DPPH): analytical pure, purchased from Sigma company, USA. Purified water (made in laboratory).

3. Test method

3.1 Flash extraction of Ligustrum lucidum seed oil
Clean and dry raw Ligustrum lucidum seed and its processed products were crushed and sifted through No. 3 sieve for standby. Weigh 200g of Ligustrum lucidum seed powder, add a certain amount of petroleum ether (60 ~ 90 ℃), set the temperature at 85 ℃, flash extraction under the set voltage, filter, recover petroleum ether from the filtrate under reduced pressure, and dry it in the oven at 70 ℃ to constant weight, the oil of Ligustrum lucidum seed is obtained [8].

3.2. Calculation method of oil yield of Ligustrum lucidum seed
The oil yield of Ligustrum lucidum seed = the quality of extracted Ligustrum lucidum seed oil / the quality of raw material of Ligustrum lucidum seed × 100%

3.3. Antioxidant activity of Ligustrum lucidum seed oil
Taking the scavenging rate of DPPH free radical and hydroxyl free radical as the preliminary evaluation index of in vitro antioxidant activity of Ligustrum lucidum seed oil, with vitamin C (Vc) as the control. The scavenging ability of DPPH radical was investigated by Ding Jinfeng et al [12]. and hydroxyl radical scavenging ability of Zhu Hongye et al [13].

3.4. Design of test method
Response surface methodology (RSM) is one of the typical methods to optimize process parameters. It can optimize process parameters with the least number of tests and analyze the interaction between process parameters. According to the results of literature review and single factor pre-test, it was found that the extraction temperature was 65 ℃, the extraction voltage was 120 ~ 160v, the liquid-solid ratio (mL/ g) was 10:1 ~ 14:1, and the extraction time was 100 ~ 200s. The oil yield fluctuated and showed significant difference, but the oil yield changed little after two times of
extraction, and there was no significant difference. Therefore, the extraction temperature was set at 70 ℃ for twice, and the oil yield of Ligustrum lucidum seed was taken as the response value. The extraction voltage, liquid-solid ratio and extraction time were optimized by response surface methodology. The code and level of test factors were shown in Table 1, and each test was parallel to 3 groups.

| Table 1. Test factors and levels. |
|-----------------------------------|
| Level | A Extraction voltage (v) | B Liquid material ratio / (mL / g) | C Extraction time / (s) |
|-------|-------------------------|-----------------------------------|------------------------|
| -1    | 120                     | 10:1                               | 100                    |
| 0     | 140                     | 12:1                               | 150                    |
| 1     | 160                     | 14:1                               | 200                    |

4. Test result

4.1 Response surface optimization analysis

Design-Expert 8.07b software is used to design the Box-Behnken response surface and analyze the data. Results the oil yield is shown in Table 2, and the analysis of variance is shown in Table 3.

| Table 2. Design and results of extraction process response surface methodology (n = 3). |
|---------------------------------------------------------------|
| Number | Extraction voltage (A / v) | Liquid material ratio (mL / g) | Extraction time (B / s) | Oil yield (%) |
|--------|---------------------------|--------------------------------|-------------------------|---------------|
| 1      | -1                        | -1                             | 0                       | 11.58         |
| 2      | 0                         | 0                              | 0                       | 13.75         |
| 3      | 0                         | 0                              | 0                       | 13.69         |
| 4      | 1                         | 1                              | 0                       | 12.07         |
| 5      | 1                         | 1                              | 0                       | 12.14         |
| 6      | 0                         | -1                             | 1                       | 11.83         |
| 7      | 1                         | 0                              | -1                      | 11.73         |
| 8      | 0                         | -1                             | -1                      | 11.62         |
| 9      | -1                        | 1                              | 0                       | 12.16         |
| 10     | 0                         | 0                              | 0                       | 13.71         |
| 11     | 0                         | 1                              | -1                      | 12.13         |
| 12     | -1                        | 0                              | -1                      | 11.92         |
| 13     | 0                         | 0                              | 0                       | 13.61         |
| 14     | -1                        | 0                              | 1                       | 12.07         |
| 15     | 1                         | 0                              | 1                       | 11.74         |
| 16     | 0                         | 0                              | 0                       | 13.59         |
| 17     | 1                         | -1                             | 0                       | 11.75         |

| Table 3. Analysis of variance. |
|---------------------------------|
| Source of variance | Sum of squares of deviations | Freedom | Mean square | F value | P           | Significance |
|--------------------|------------------------------|---------|-------------|---------|-------------|--------------|
| Model              | 10.58                        | 9       | 1.18        | 56.90   | <0.0001     | Extremely significant |
| A                  | 0.017                        | 1       | 0.017       | 0.83    | 0.3930      |              |
| B                  | 0.37                         | 1       | 0.37        | 17.90   | 0.0039      | Extremely significant |
| C                  | 0.012                        | 1       | 0.012       | 0.58    | 0.4706      |              |
| AB                 | 9.025E-003                   | 1       | 9.025E-003  | 0.44    | 0.5297      |              |
The quadratic multiple regression equation obtained from the data in Table 2 is as follows:

\[ Y = 13.59 - 0.046A + 0.22B + 0.039C - 0.048AB - 0.035AC - 0.068BC - 0.87A^2 - 0.82B^2 - 0.86C^2 \]

Table 3. Analysis of variance results show that the regression model \( P < 0.0001 \), with highly significant, and \( R^2 = 0.9865 \), mismatch item \( P > 0.05 \) has no significant, indicating that this model has a good fitting degree with the actual test, \( =0.9865 \), the one degree term B is highly significant, in the quadratic term, \( A^2, B^2 \) and \( C^2 \) were highly significant, and the interaction of \( A, B \) and \( C \) was not significant, and the order of influence on extraction process was: \( B > A > C \). The results show that the method is feasible for the prediction and analysis of the flash extraction process of Ligustrum lucidum seed oil. The 3D effect picture is shown in Figure 1.

4.2. Verification test

The optimal extraction process was as follows: under these conditions, the extraction voltage was 139.4v, the ratio of liquid to material was 12.26:1, and the extraction time was 151s. Under these conditions, the oil yield of Ligustrum lucidum seed was 13.61%. Under these conditions, the oil yield of Ligustrum lucidum seed was 13.61%. In order to test the validity of the regression model and consider the convenience of operation, the optimal conditions obtained by response surface quadratic regression were modified. The optimal extraction process was determined as follows: extraction voltage 140 v, liquid-solid ratio 12:1, extraction time 150s. The results show that the average oil yield is 13.53%, which is close to the predicted value. It shows that the method fits well with the actual situation, and the regression model is effective. Therefore, the optimization of flash extraction process of Ligustrum lucidum seed oil by response surface methodology is reliable and has practical value.

4.3. Results and analysis of antioxidant activity of Ligustrum lucidum seed oil

4.3.1. DPPH radical scavenging capacity. The results showed that Ligustrum lucidum seed oil had a certain DPPH · free radical scavenging ability, and its DPPH · free radical scavenging ability was positively correlated with its mass concentration. When the concentration of Ligustrum lucidum seed oil was 2.5 mg / mL, the clearance rate was 32.25%. However, the scavenging rate of VC on
DPPH · free radical in control group was significantly higher than that in Ligustrum lucidum seed oil, reaching 94.12%. The scavenging rate of Vc on DPPH · free radical had no correlation with the mass concentration, and its scavenging capacity was basically unchanged. The results are shown in Fig. 2.

4.3.2. Hydroxyl radical scavenging capacity. The results showed that Ligustrum lucidum seed oil also had high scavenging ability to hydroxyl radical, and the scavenging ability was positively correlated with the mass concentration. When the mass concentration was 2.5mg/mL, the scavenging rate was 43.83%. However, compared with Vc control group, the clearance rate was significantly lower than that of Vc control group. The results are shown in Fig. 3.

5. Conclusion
In this study, taking the yield of Ligustrum lucidum seed oil as the index, the flash extraction method was used to extract the Ligustrum lucidum seed oil. On the basis of the pre-test, the response surface method and variance analysis were used to optimize the extraction process parameters. The results showed that the optimal extraction conditions of Ligustrum lucidum seed oil were as follows: liquid-solid ratio 12:1, voltage 140v, extraction time 150s. The results showed that the response surface method was more accurate and direct than the traditional method to obtain the best process
parameters, and the average oil yield was 13.53%. At the same time, the antioxidant activity of Ligustrum lucidum seed oil in vitro was analyzed. The results showed that Ligustrum lucidum seed oil had strong antioxidant activity, but the activity was significantly lower than Vc. When the mass concentration was 2.5 mg/mL, the scavenging rate of DPPH free radical and hydroxyl radical was 32.25% and 43.83% respectively.

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