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Optimization of Steam Distillation Process for Volatile Oils from *Forsythia suspensa* and *Lonicera japonica* According to the Concept of Quality by Design

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Abstract: In this study, the process of steam distillation to collect volatile oils from *Forsythia suspensa* (*F. suspensa*) and *Lonicera japonica* (*L. japonica*) was optimized according to the concept of quality by design. First, the liquid/material ratio, distillation time, and collection temperature were identified as critical process parameters by a review of the literature and single-factor experiments. Then, a Box–Behnken design was used to study the quantitative relationship between the three process parameters, two raw material properties, and the yield of volatile oil. A mathematical model was established with an R^2 value exceeding 0.90. Furthermore, the design space of the volatile oil yield was calculated by a probability-based method. The results of a verification experiment showed that the model was accurate and the design space was reliable. A total of 16 chemical constituents were identified in the volatile oil from mixtures of *F. suspensa* and *L. japonica*. The content of β-pinene was the highest (54.75%), and the composition was similar to that of the volatile oil of *F. suspensa*. The results showed that when *F. suspensa* and *L. japonica* were distilled together, the main contribution to the volatile oil was from *F. suspensa*. The volatile oil yield from the combination of *F. suspensa* and *L. japonica* was not higher than that from *L. japonica*.

Keywords: *Forsythia suspensa*; *Lonicera japonica*; volatile oil; steam distillation; design space

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1. Introduction

Forsythia suspensa is the dry fruit of *Forsythia suspensa (Thunb.) Vahl*, and *Lonicera japonica* consists of the dry buds or flowers of *Lonicera japonica Thunb.* that bloom early in the spring [1]. Both have the medicinal functions of reducing fevers, detoxification, and dispersing wind-heat and are often used together as a pair in Chinese medicine [2–4]. The 2020 edition of the *Chinese Pharmacopoeia* lists 45 Chinese patent medicines that use *F. suspensa* and *L. japonica* in combination [1]. The volatile oils of *F. suspensa* and *L. japonica* are collected and used in the manufacture of four Chinese patent medicines, including Xiao'er Resuqing Koufuye, Xiao'er Resuqing Keli, Xiao'er Resuqing Tangjiang, and Jinchan Zhiyang Jiaonang [1]. At present, several studies have shown that the volatile oil of *F. suspensa* has antibacterial, antioxidant, antiviral, antipyretic, anti-inflammatory, antitumor, and other pharmacological effects [3,5,6]. The volatile oil of *L. japonica* also has pharmacological effects, such as heat clearing, detoxification, and antibacterial activity [2,4,7].

Steam distillation is a commonly used method to collect volatile oils [8–13], and it has the advantages of simple equipment, easy operation, low cost, and the use of safe solvents [14–16]. However, it is also a time-consuming process with a low volatile oil

yield. The steam distillation process is used in the production of the abovementioned four Chinese patent medicines from the volatile oils collected from *F. suspensa* and *L. japonica* [1].

References from the literature concerning the distillation of the volatile oils of *F. suspensa* or *L. japonica* by steam distillation are listed in Table 1. Liu Yan et al. [17] and Wang Yan et al. [18] studied the three parameters of soaking time, distillation time, and material/liquid ratio and found that the influence of each parameter on the volatile oil yield was: material/liquid ratio > distillation time > soaking time. The volatile oil yields from *F. suspensa* and *L. japonica* were approximately 0.93 mL/100 g and 0.34 mL/100 g, respectively. Gu Ke et al. [19] and Li Jianjun et al. [20] studied the collection of volatile oil from *L. japonica* by steam distillation, and the volatile oil yields were about 0.15 g/100 g and 0.17 g/100 g. However, Tong Qiaozhen et al. [21] failed to distillate volatile oil from *L. japonica* by steam distillation. In these published works, the authors suggested that the volatile oil of *L. japonica* has a relatively high solubility in water, which may lead to the loss of *L. japonica* volatile oil in the steam distillation process. When co-distilled with *F. suspensa*, the volatile oil of *F. suspensa* may extract some *L. japonica* volatile oil from the aqueous phase, resulting in the collection of more *L. japonica* volatile oil. However, at present, there are no references in the literature addressing the steam distillation of volatile oil from mixtures of *F. suspensa* and *L. japonica*. Therefore, the authors tried to obtain the volatile oil by the co-distillation of *F. suspensa* and *L. japonica*.

Table 1. Literature on steam distillation.

Medicinal Material	Design of Experiment	Material/Liquid Ratio (g:mL)	Soaking Time (h)	Distillation Time (h)	Optimum Conditions	Volatile Oil Yield	Reference
F. suspensa	Taguchi design	1:8~1:12	1~4	2~6	Material/liquid ratio 1:8, soaking time 2 h, distillation time 6 h.	0.926 mL/100 g	[17]
F. suspensa	Box–Behnken design	1:4~1:10	8~24	8~20	Material/liquid ratio 1:5, soaking time 21 h, distillation time 11 h.	0.342 mL/100 g	[18]
L. japonica	Single-factor design, Box–Behnken design	1:15~1:25	0~5	7~11	Material/liquid ratio 1:20, soaking time 1 h, distillation time 7 h.	0.14998 g/100 g	[19]
L. japonica	Single-factor design, Taguchi design	1:9~1:11	20~28	38~42	Material/liquid ratio 1:10, soaking time 28 h, distillation time 42 h	0.171 g/100 g	[20]

In recent years, the concept of quality by design [22–25] has been used in the optimization of many processes related to Chinese medicines, such as distillation [26], precipitation [27], and column chromatography [28]. In this study, based on the concept of quality by design, the steam distillation process was optimized to distill the volatile oils of *F. suspensa* and *L. japonica*. One of the aims of this work was to find the optimized parameters of steam distillation for collecting a relatively stable amount of volatile oil, which would improve the batch-to-batch consistency of Chinese medicine quality. The critical process parameters were determined through a search of the literature and single-factor experiments. The critical raw material properties of *F. suspensa* were also determined. The quantitative relationship between the process parameters, raw material properties, and volatile oil yield was studied by a Box–Behnken design [29], and a mathematical model was established. The design space of the distillation process of volatile oil from *F. suspensa* and *L. japonica* was calculated by a probability-based method. The operation points inside and outside the design space were selected for verification. Finally, the chemical composition of the volatile oil of *F. suspensa* and the volatile oil of mixtures of *F. suspensa* and *L. japonica* were analyzed and compared.

2. Materials and Methods

2.1. Materials and Reagents

Methanol (chromatographically pure) was obtained from Merck, Germany. Acetonitrile (chromatographically pure) was also obtained from Merck, Germany. Ultrapure water was prepared by an ultrapure water preparation system (Milli-Q, Millipore, Germany). β-Pinene (batch number: C12071236, purity ≥ 98%) was purchased from Shanghai McLean Biochemical Technology Co., Ltd. The medicinal material numbers, origins, and suppliers of *F. suspensa* and *L. japonica* are shown in Table 2.

Table 2. Origin and batch information of medicinal materials.

Medicinal Materials	Number	Origin	Companies
L. japonica	JYH-1	Shandong	Zhejiang Huqing Yutang Materia Medica Co., Ltd.
	JYH-2	Hebei	Hebei Linyitang Pharmaceutical Co., Ltd.
F. suspensa	LQ-1	Shanxi	Haozhou Feimao Pharmaceutical Co., Ltd.
	LQ-2	Shaanxi	Haozhou Chujian Huakai E-Commerce Co., Ltd.
	LQ-3	Shanxi	Nanjing Shangyuantang Pharmaceutical Co., Ltd.
	LQ-4	Shaanxi	Anguo Pharmaceutical Source Trading Co., Ltd.
	LQ-5	Shanxi	Hebei Linyitang Pharmaceutical Co., Ltd.
	LQ-6	Gansu	Sichuan Xunbai Herbal Industry Co., Ltd.

2.2. Steam Distillation

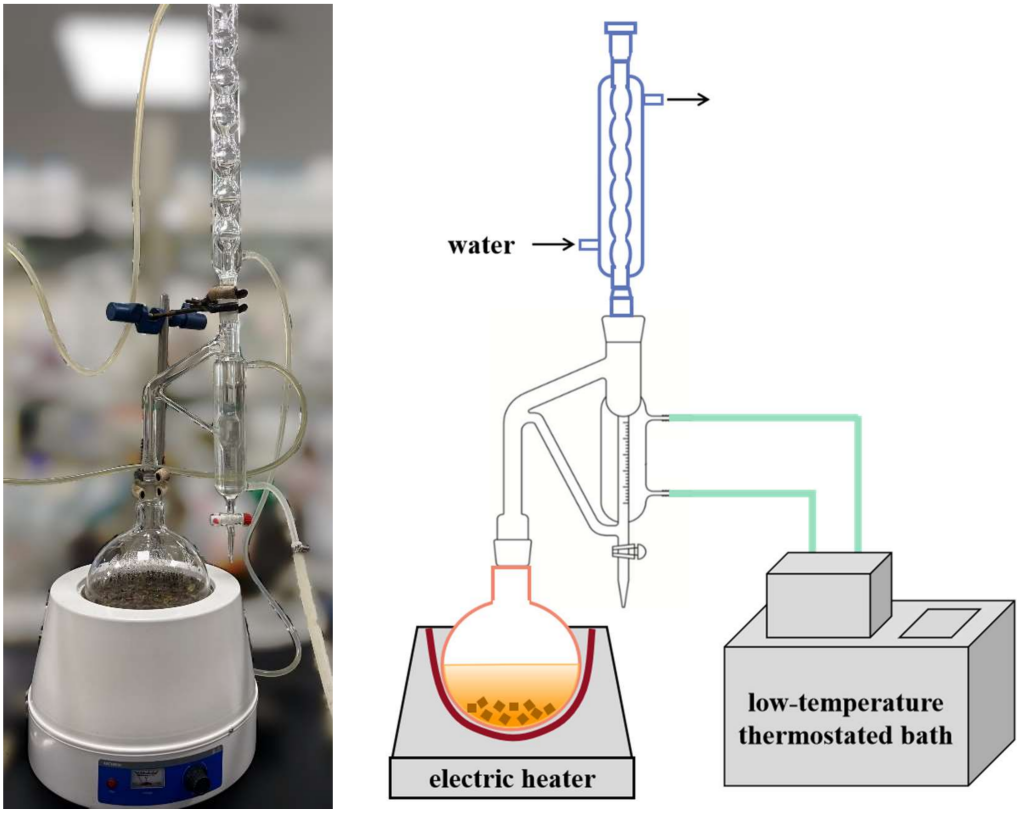
The volatile oil in *F. suspensa* and *L. japonica* was collected by steam distillation. The experimental setup is shown in Figure 1. A low-temperature thermostated bath (THYD-1030 W, Ningbo Tianheng Instrument Factory) was used to lower the temperature of the collection part of the volatile oil extractor [30]. An electronic balance (CN-LQC60002, Kunshan Youkeweiter Electronic Technology Co., Ltd.) was used to weigh 50 g of *F. suspensa* and 50 g of *L. japonica* (Medicinal Material Number: JYH-2). The samples were placed in a 2000 mL flask. A certain amount of water was added, and the flask was shaken to fully wet the medicinal materials. The volatile oil extractor and the condenser pipe were connected. The condensed glycerol and condensed water were fed into the water-cooling jacket and the condenser pipe of the volatile oil extractor, respectively. The volatile oil extractor was filled with water from the top of the condenser until it overflowed into the flask. The electric heater (DZTW 2000 mL, Shaoxing Yuecheng Kechen Instrument and Equipment Co., Ltd.) was turned on, and it started slowly heating the water to boiling. After boiling began, the power of the electric heater was adjusted to 50 W. Starting with the first drop of condensed water dripping into the volatile oil extractor, heating was stopped after heating and refluxing for a certain period of time. After the volatile oil stood for 10 min, its volume was recorded. The volatile oil yield was calculated as shown in Formula (1).

$$\text{Volatile oil yield (mL/100 g)} = \text{volatile oil volume (mL)/medicinal material (100 g)} \quad (1)$$

2.3. Determination of the Volatile Oil Content in F. suspensa

The thermostat was used to lower the temperature of the collection part of the volatile oil extractor [30]. An electronic balance was used to weigh 100 g of *F. suspensa*, which was then placed in a 2000 mL flask. A certain amount of water was added, and the flask was shaken to fully wet the medicinal materials. The volatile oil extractor and the condenser pipe were connected. The condensed glycerol and condensed water were fed into the water-cooling jacket and the condenser pipe of the volatile oil extractor, respectively. The volatile oil extractor was filled with water from the top of the condenser until it overflowed into the flask. The electric heater was turned on, and it slowly heated the water to boiling. After boiling, the power of the electric heater was adjusted to 50 W. Starting with the first drop of condensed water dripping into the volatile oil extractor, heating was stopped after heating and refluxing for a certain period of time. After the volatile oil stood for 10 min, its

volume was recorded. The volatile oil content (mL/100 g) in different batches of *F. suspensa* was calculated as shown in Formula (1).



(**a**) Photograph (**b**) Schematic diagram

Figure 1. Experimental device for steam distillation.

2.4. Determination of β-Pinene Content in F. suspensa

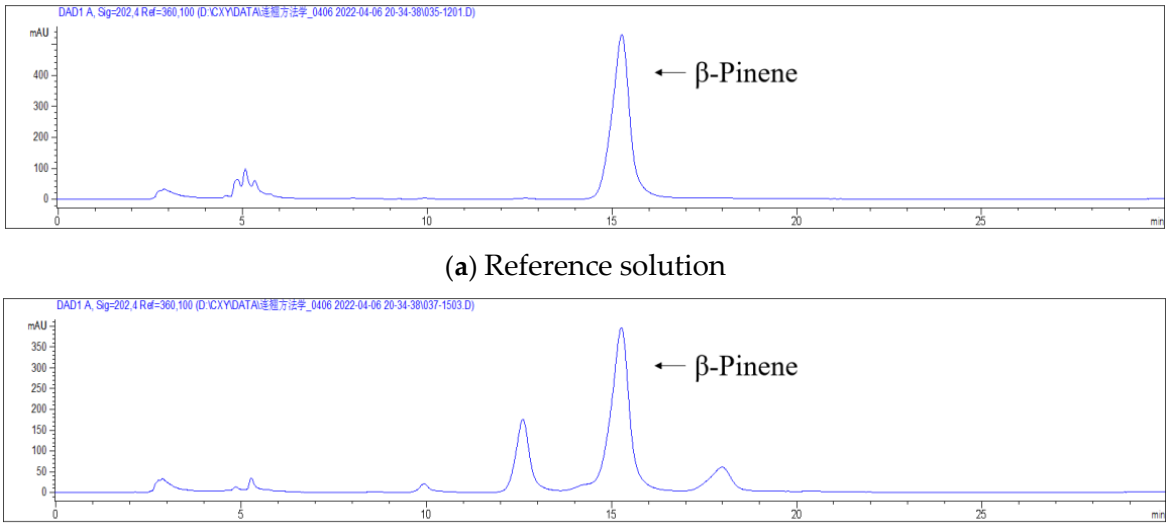
2.4.1. Preparation of Reference Solution and Test Solution

Because the reference substance of β-pinene was a liquid, we precisely measured 83.40 mg of the β-pinene reference substance with a pipette. After that, it was diluted to 10 mL with methanol to obtain the storage reference solution with a concentration of 8340 μg/mL. Then, the storage reference solution was diluted 50, 100, 200, 400, 500, and 800 times with methanol, respectively, to prepare reference solutions with concentrations of 166.8, 83.40, 41.70, 20.85, 16.68, and 10.42 μg/mL.

One hundred microliters of the distilled *F. suspensa* volatile oil was precisely measured, placed in a 10 mL volumetric flask, diluted to volume with methanol, and shaken well to obtain the concentrated stock solution of the test product. A total of 100 μL of the concentrated stock solution of the test product was precisely measured, placed in a 5 mL volumetric flask, diluted to volume with methanol, and shaken well to obtain the test solution.

2.4.2. Chromatographic Conditions

The β-Pinene content in the volatile oil was determined using liquid chromatography (Agilent 1100-DAD, Agilent, USA). The HPLC conditions were modified from the method described in the literature [31]. The details were as follows: Column—Agilent ZORBAX SB-C18, 4.6×250 mm (5-Micron). Mobile phase—0.4% phosphoric acid solution (A)/acetonitrile (B), gradient elution (0 min, 65% B; 0~5 min, 65~70% B; 5~25 min, 70% B; 25~30 min, 70~80% B). Post-run—15 min; injection volume—10 μL; flow rate—1 mL/min; column temperature—25 °C; detection wavelength—202 nm. The chromatograms of the reference solution and test solution are shown in Figure 2.



(**a**) Reference solution

(**b**) Test solution

Figure 2. HPLC chromatograms of β-pinene.

2.4.3. Methodological Validation

Linear relationship investigation: The β-pinene reference substance was used to investigate the linear relationship. Reference solutions with concentrations of 166.8, 83.40, 41.70, 20.85, 16.68, and 10.42 μg/mL were prepared according to the method described in Section 2.4.1. A 0.22 μm microporous filter membrane was used for filtration, injection, and analysis according to the chromatographic conditions described in Section 2.4.2. Taking the concentration of the reference solution as the abscissa (X, μg/mL) and the peak area as the ordinate (Y, mAU), the linear regression equation and correlation coefficient were obtained.

Precision test: The volatile oil of *F. suspensa* was used to prepare the concentrated stock solution of the test product according to the method outlined in Section 2.4.1. One milliliter of the concentrated stock solution of the test product was accurately absorbed, placed in a 10 mL volumetric flask, diluted to volume with methanol, and shaken well to obtain the test solution. The test solution was filtered with a 0.22 μm microporous membrane. The samples were injected into the column 6 times continuously according to the chromatographic conditions provided in Section 2.4.2, and the RSD value of the peak area was calculated.

Repeatability test: Six replicates of the same batch of *F. suspensa* volatile oil were obtained. The concentrated stock solution of the test product was prepared according to the method in Section 2.4.1. One milliliter of the concentrated stock solution of the test product was accurately absorbed, placed in a 10 mL volumetric flask, diluted to volume with methanol, and shaken well to obtain the test solution. The test solution was filtered with a 0.22 μm microporous membrane. The samples were injected and analyzed according to the chromatographic conditions outlined in Section 2.4.2, and the RSD value of the concentration was calculated.

Stability test: The volatile oil of *F. suspensa* was used to prepare a concentrated stock solution of the test product according to the method described in Section 2.4.1. One milliliter of the concentrated stock solution of the test product was accurately measured, placed in a 10 mL volumetric flask, diluted to volume with methanol, and shaken well to obtain the test solution. The test solution was filtered with a 0.22 μm microporous membrane. The samples were injected and analyzed at 0, 3, 6, 9, 12, and 24 h according to the chromatographic conditions described in Section 2.4.2, and the relative standard deviation (RSD) value of the peak area was calculated.

Sample addition and recovery test: We took 9 aliquots of *F. suspensa* volatile oil from the same batch with a known content of β-pinene and prepared a concentrated stock solution of the test sample according to the method described in Section 2.4.1. One milliliter

of the concentrated stock solution of the test sample was accurately absorbed, placed in a 20 mL volumetric flask, diluted to volume with methanol, and shaken well to obtain the test solution (33.2 μg/mL). The reference solution (41.7 μg/mL) was prepared according to the method outlined in Section 2.4.1. The test solution was divided into three groups: low, medium, and high. We precisely measured 1.25 mL of each group and placed it in a 5 mL volumetric flask. The ratios of the added amount of the reference substance to the content of β-pinene in the test solution were controlled at approximately 0.5:1.0, 1.0:1.0, and 1.5:1.0, and 0.5, 1.0, and 1.5 mL of the reference substance was added to the low, medium, and high groups, respectively. Each mixture was diluted to volume with methanol, shaken well, and filtered with a 0.22 μm microporous membrane. The samples were injected and analyzed according to the chromatographic conditions provided in Section 2.4.2, and the β-pinene content and sample recovery rate of each group were calculated.

2.4.4. Determination of Content

An appropriate amount of volatile oil was taken from different batches of *F. suspensa*. The test solutions were prepared according to the method outlined in Section 2.4.1. Then, the test solutions were injected and analyzed according to the chromatographic conditions described in Section 2.4.2. The peak areas were recorded, and the β-pinene contents in different batches of *F. suspensa* were calculated.

2.5. Optimization of Distillation Process Parameters

Based on the literature search and single-factor experiments, it was believed that the critical process parameters affecting the volatile oil yield from *F. suspensa* and *L. japonica* by steam distillation were the amount of water, distillation time, and collection temperature, and the critical properties of the raw materials were the volatile oil content and the β-pinene content of *F. suspensa*.

A Box–Behnken design was adopted, and the water addition (X_1), distillation time (X_2), collection temperature (X_3), volatile oil content (Z_1), and β-pinene content (Z_2) were used as the investigation factors. The volatile oil yield (Y) was used as the evaluation index to optimize the distillation process of volatile oil from *F. suspensa* and *L. japonica*. The factors and levels of the Box–Behnken design are shown in Table 3, and the results are shown in Table 4.

2.6. Analysis of Chemical Constituents of Volatile Oil

A combined total of 20 μL of volatile oil from *F. suspensa* and volatile oil from *F. suspensa* and *L. japonica* was accurately measured; then, the samples were supplemented with 1 mL of diethyl ether for dilution, filtered with a 0.22 μm microporous membrane, and placed into sample bottles. The chemical constituents in the volatile oil of *F. suspensa* and the volatile oil of *F. suspensa* and *L. japonica* were analyzed.

Table 3. The factors and levels of the Box–Behnken design.

Factor	Level		
	Low (−1)	**Medium (0)**	**High (1)**
X_1: water addition (mL/g)	8	10	12
X_2: distillation time (h)	3.0	4.5	6.0
X_3: collection temperature (°C)	5	15	25

Table 4. The results of the Box–Behnken design.

No	X_1: Water Addition (mL/g)	X_2: Distillation Time (h)	X_3: Collection Temperature (°C)	Z_1: Volatile Oil Content (mL/100 g)	Z_2: β-Pinene Content (mg/g)	Y: Volatile Oil Yield (mL/100 g)
1	8	3.0	15	1.601	0.857	0.63
2	12	3.0	15	1.599	0.867	0.54
3	8	6.0	15	1.792	1.039	0.92
4	12	6.0	15	1.349	0.812	0.83
5	8	4.5	5	1.969	1.139	0.90
6	12	4.5	5	1.601	0.857	0.80
7	8	4.5	25	1.599	0.867	0.80
8	12	4.5	25	1.792	1.039	0.83
9	10	3.0	5	1.349	0.812	0.74
10	10	6.0	5	1.969	1.139	0.98
11	10	3.0	25	1.601	0.857	0.61
12	10	6.0	25	1.599	0.867	0.71
13	10	4.5	15	1.792	1.039	0.82
14	10	4.5	15	1.349	0.812	0.80
15	10	4.5	15	1.969	1.139	0.89
16	10	4.5	15	1.969	1.139	0.94
17	10	4.5	15	1.969	1.139	0.90

The chemical constituents of the volatile oils were analyzed with a gas chromatography–mass spectrometer [32,33] (GC–MS; Agilent 7890B-7000C, Agilent, USA). The GC–MS conditions were modified from the methods describe in the literature [34,35]. The conditions were as follows: chromatographic column—Agilent HP-5MS, 30 m × 0.25 mm × 0.25 μm; inlet temperature—250 °C; flow—1 mL/min constant flow (He); split ratio—10:1; heating program—40 °C for 4 min, 2 °C/min to 100 °C, hold for 10 min, 10 °C/min to 200 °C, and hold for 5 min; MS detector—detector temperature 250 °C and scan range m/z 30–550.

2.7. Data Processing

2.7.1. Mathematical Model

The results of the Box–Behnken design were analyzed using Design-Expert 12.0.3 software (American Stat-Ease Company). Taking the volatile oil yield (Y) as the evaluation index, quadratic polynomial fitting was performed on the five factors of water addition (X_1), distillation time (X_2), collection temperature (X_3), volatile oil content (Z_1) and β-pinene content (Z_2). The mathematical model is shown as Formula (2).

$$Y = a_0 + \sum_{i=1}^{n} b_i X_i + \sum_{i=1}^{n} b_{ii} X_i^2 + \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} b_{ij} X_i X_j + \sum_{k=1}^{m} d_k Z_k \quad (2)$$

where X is a process parameter; Z is a raw material property; Y is the evaluation index; superscripts n and m are the number of process parameters and raw material properties, respectively; a_0 is the intercept; and b and d are the partial regression coefficients. The model was simplified using a stepwise regression method with p values of 0.1 for both inclusion and removal from the model.

2.7.2. Design Space

The construction design space was calculated by the probability-based method using MATLAB R2018b (American Math Works Company). Under the conditions of fixed batches of medicinal materials, the effects of the changes in the three process parameters on the volatile oil yield were randomly simulated. The parameter combination with a lower limit of volatile oil yield of 0.60 mL and a probability of reaching the standard of no less than 0.80 was used as the design space. In the calculation, the steps of water addition, distillation time, and collection temperature were set to 0.04, 0.03, and 0.10, respectively. The number of simulations was 1000.

3. Results

3.1. Single-Factor Experiments

The results of the single-factor experiments are shown in Table 5. The results indicated that the effects of crushed particle size and soaking time on the volatile oil yield were not significant. The volatile oil yield decreased with increasing water addition, which may have been due to the increase in water improving the amount of volatile oil dissolved in water, which resulted in a decrease in the volatile oil yield.

Table 5. Single-factor experiments.

Factor	Medicine Number	Particle Size	Soaking Time (h)	Water Addition (mL/g)	Distillation (h)	Volatile Oil Yield (mL/100 g)
Particle size	LQ-5	Not crushed Coarsest flour Coarse flour	0	10	5	1.900 1.820 1.827
Soaking time	LQ-5	Not crushed	0 2 4	10	5	1.900 1.922 1.952
Water addition	LQ-1	Not crushed	0	8 10 12	5	1.485 1.479 1.350

Table 6 shows the results of separately distilling volatile oils from *F. suspensa* and *L. japonica*. The results showed that no volatile oil was distilled from the two different batches of *L. japonica*, which may have been because the *L. japonica* used in this study contained little volatile oil. *F. suspensa* could be distilled to obtain volatile oil. Therefore, in a follow-up study, we will investigate whether the distillation of volatile oil from *L. japonica* can be promoted by the combined steam distillation of *F. suspensa* and *L. japonica*.

Table 6. Separate distillation of volatile oils from *F. suspensa* or *L. japonica*.

Medicine Number	Water Addition (mL/g)	Distillation Time (h)	Collection Time (°C)	Volatile Oil Yield (mL/100 g)
LQ-5	10	5	5	1.864
JYH-1	10	5	5	0
JYH-2	10	5	5	0

The results of the co-distillation of volatile oil from *F. suspensa* and *L. japonica* are shown in Table 7. The effects of water addition and collection temperature on the volatile oil yield were investigated. The results showed that both had a certain influence on the volatile oil yield.

Table 7. Co-distillation of volatile oils from *F. suspensa* and *L. japonica*.

Medicine Number		Water Addition (mL/g)	Distillation Time (h)	Collection Temperature (°C)	Volatile Oil Yield (mL/100 g)
LQ-5	JYH-2	10	5	Indoor temperature	0.930
		12	5	Indoor temperature	0.851
LQ-1	JYH-2	10	5	5	0.880
		12	5	5	0.930

3.2. Methodological Validation

Taking the concentration of the β-pinene reference solution as the abscissa (X, μg/mL) and the corresponding peak area as the ordinate (Y, mAU), linear regression was performed

to obtain the linear regression equation: Y = 210.56X + 282.59. The linear range of β-pinene was 10.425–166.8 μg/mL, and the coefficient of determination R^2 in this range was 0.9997, which confirmed a good linear relationship. The results of the precision test showed that the RSD of the peak area of β-pinene was 0.68%, indicating that the precision of the instrument was good. The results of the repeatability test showed that the RSD of the β-pinene concentration was 0.84%, which proved that the method had good repeatability. The results of the stability test showed that the peak-area RSD of β-pinene was 1.84%, indicating that the test solution had good stability within 24 h. The results of the sample addition recovery test are shown in Table S1. The measured recovery rates were between 95 and 102%, the average recovery rate was 98.71%, and the RSD of the three levels was 1.89%, which met the measurement requirements, indicating that the method has good accuracy.

3.3. Characterization of Raw Material Properties of Different Batches of F. suspensa

The process parameters of the steam distillation method were fixed as follows: 100 g raw material, 1000 mL water addition, 5 °C collection temperature, and 6 h distillation time. The contents of volatile oil and β-pinene in different batches of *F. suspensa* were measured as shown in Table 8. The results showed that the raw material properties of different batches of *F. suspensa* were quite different. For example, the volatile oil content of LQ-4 was 1.394 mL/100 g, while the volatile oil content of LQ-5 was 1.969 mL/100 g. The β-pinene content of LQ-6 was 0.746 mg/100 g, while the β-pinene content of LQ-5 reached 1.139 mg/100 g.

Table 8. Characterization of raw material properties of different batches of *F. suspensa*.

Medicine Number	Volatile Oil Content (mL/100 g)	β-Pinene Content (mg/100 g)
LQ-1	1.601	0.857
LQ-2	1.599	0.867
LQ-3	1.792	1.039
LQ-4	1.394	0.812
LQ-5	1.969	1.139
LQ-6	1.500	0.746

3.4. Optimization of Distillation Parameters of Volatile Oil

3.4.1. Data Processing and Model Fitting

The polynomial regression model obtained by modeling the data acquired from the experimental distillation of volatile oil from *F. suspensa* and *L. japonica* is shown in Formula (3).

$$Y = 0.2294 + 0.04977 \times 2 - 0.5199Z_1 + 1.325Z_2 - 0.000318 \times X_1X_3 \quad (3)$$

The coefficient of determination of the model R^2 was 0.9022, indicating that the model fit well and could explain the changes in the experimental data. The variance analysis of each item in the model is shown in Table 9. The p value of the model was less than 0.0001, indicating that the model was extremely significant. In the model, X_2 ($p = 0.0067$), Z_1 ($p = 0.0014$), and Z_2 ($p = 0.0001$) were all extremely significant items, indicating the influence of distillation time and raw material properties (volatile oil content, β-pinene content) on the volatile oil yield. X_1X_3 ($p = 0.0139$) was also a significant item, which indicated that the interaction of the two factors of water addition and collection temperature was significant for volatile oil yield.

Table 9. Regression coefficient and variance analysis of the model.

Factor	Y	
	Coefficient	***p* Value**
Constant	0.2294	
X_2	0.04977	0.0067 **
Z_1	−0.5199	0.0014 **
Z_2	1.325	0.0001 **
X_1X_3	−0.000318	0.0139 *
Model *p* value	<0.0001	
R^2	0.9022	
R^2_{adj}	0.8695	

* $p < 0.05$, ** $p < 0.01$.

3.4.2. Contour Diagram

The contour maps of the volatile oil yield from *F. suspensa* and *L. japonica* are shown in Figures 3–5. The figures reflect the effect of water addition, distillation time, and collection temperature on the volatile oil yield under the conditions of fixed raw material properties. Under the conditions of a constant collection temperature with increasing distillation time, the volatile oil yield increased gradually. Under the conditions of a constant distillation time with increasing water addition and a decreasing collection temperature, or decreasing water addition and an increasing collection temperature, the volatile oil yield increased. Under the conditions of unchanging water addition with increasing distillation time, the volatile oil yield increased gradually.

3.4.3. Design Space Calculation and Verification

The properties of the raw materials were fixed as LQ-6, and the design space calculated by the probability-based method is shown in Figures 6 and 7. The design space diagram shows that the distillation time had the greatest influence on the volatile oil yield.

A point inside the design space and a point outside the design space were selected for verification. The selection conditions of the verification points in the design space were as follows: the water addition was 10 mL/g, the distillation time was 6 h, and the collection temperature was 10 °C. The probability of reaching the standard was 0.976. Under these conditions, the volatile oil yield predicted by the model was 0.71 mL. According to the above conditions, three parallel experiments were carried out. The obtained volatile oil yields were 0.76 mL, 0.71 mL, and 0.74 mL, respectively. The average volatile oil yield was 0.74 mL, and the relative standard deviation was 2.52%.

The selection conditions of the verification point outside the design space were as follows: the water addition was 10 mL/g, the distillation time was 3 h, and the collection temperature was 10 °C. The probability of reaching the standard was 0.333. Under these conditions, the volatile oil yield predicted by the model was 0.56 mL, and the experimental results were 0.60 mL, 0.60 mL, and 0.58 mL, respectively. The average volatile oil yield was 0.59 mL, and the relative standard deviation was 1.15%. The measured values of the two verification points were relatively close to the model-predicted values, indicating that the mathematical model established according to the Box–Behnken design was accurate. The volatile oil yield at the verification point in the design space was higher than the preset standard, and the volatile oil yield at the verification point outside the design space was lower than the preset standard, indicating that the design space was reliable.

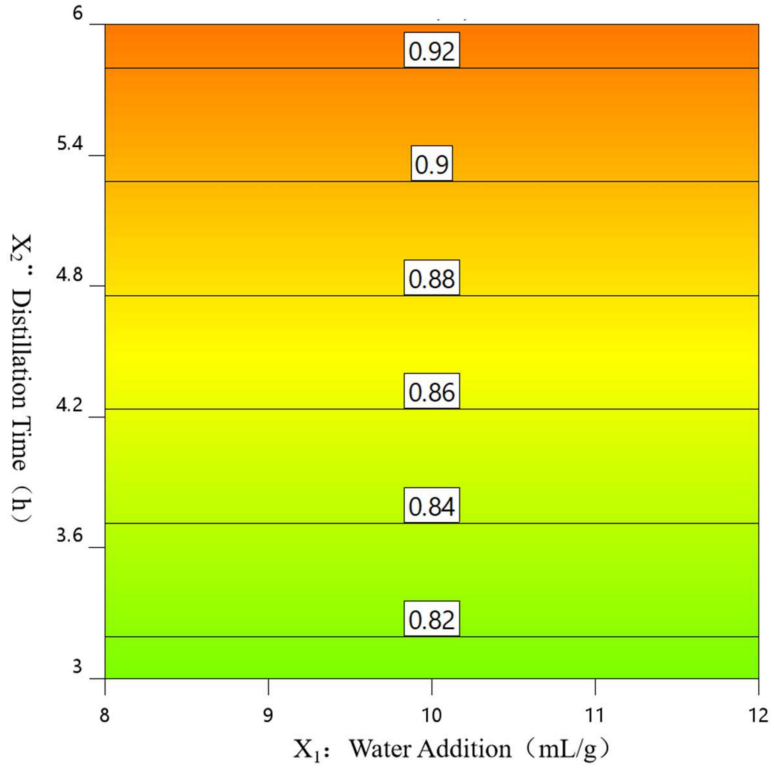


Figure 3. Volatile oil yield at a fixed collection temperature of 10 °C.

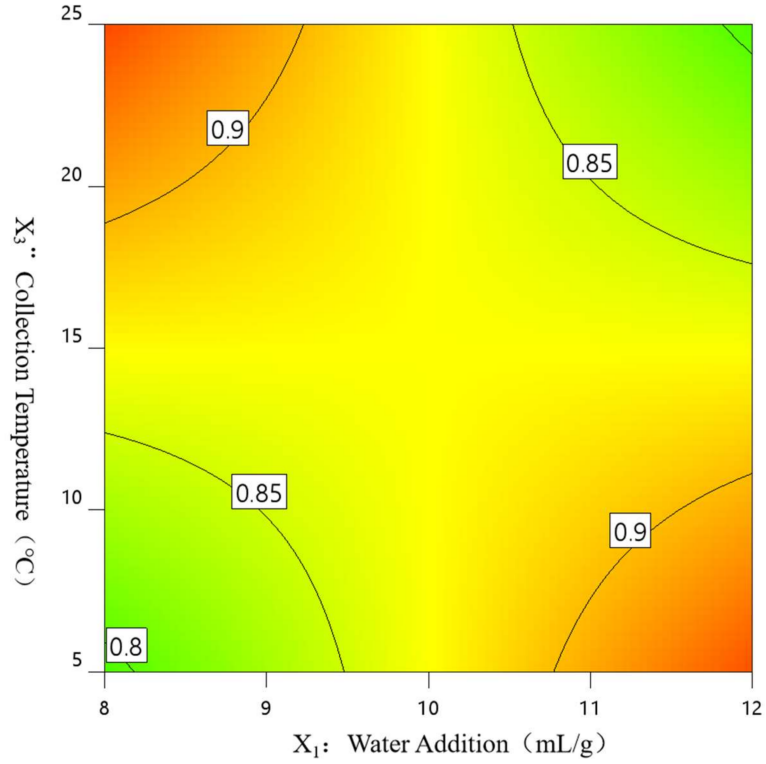


Figure 4. Volatile oil yield when the fixed distillation time was 4.5 h.

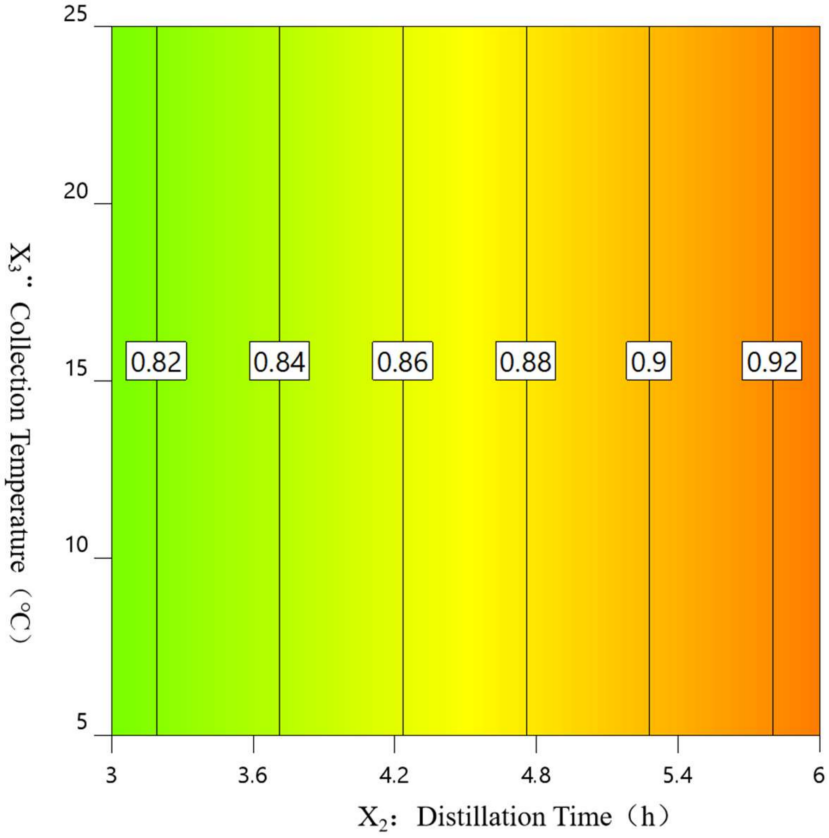


Figure 5. Volatile oil yield when the fixed water addition was 10 mL/g.

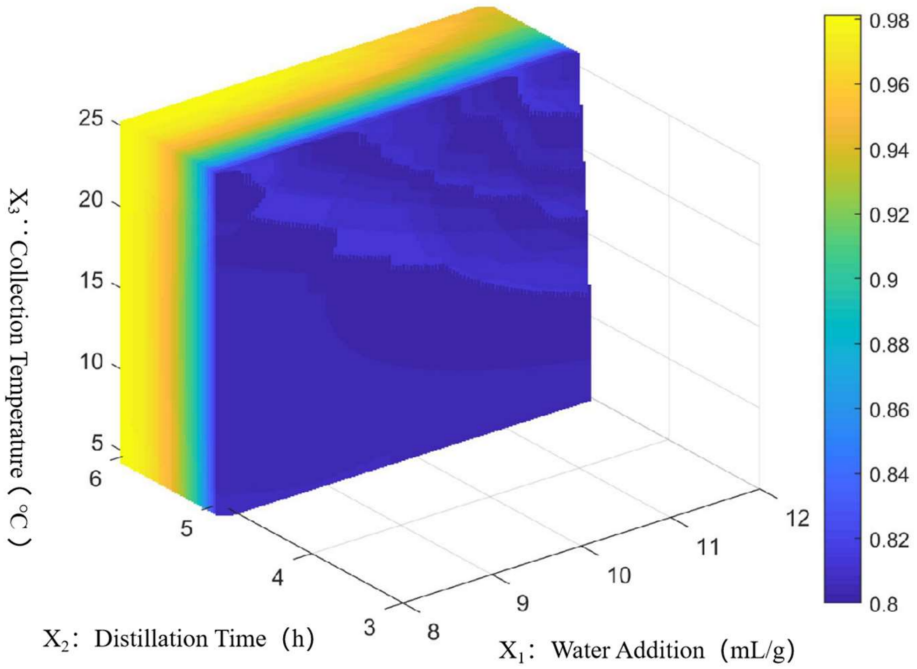


Figure 6. Three-dimensional design space diagram.

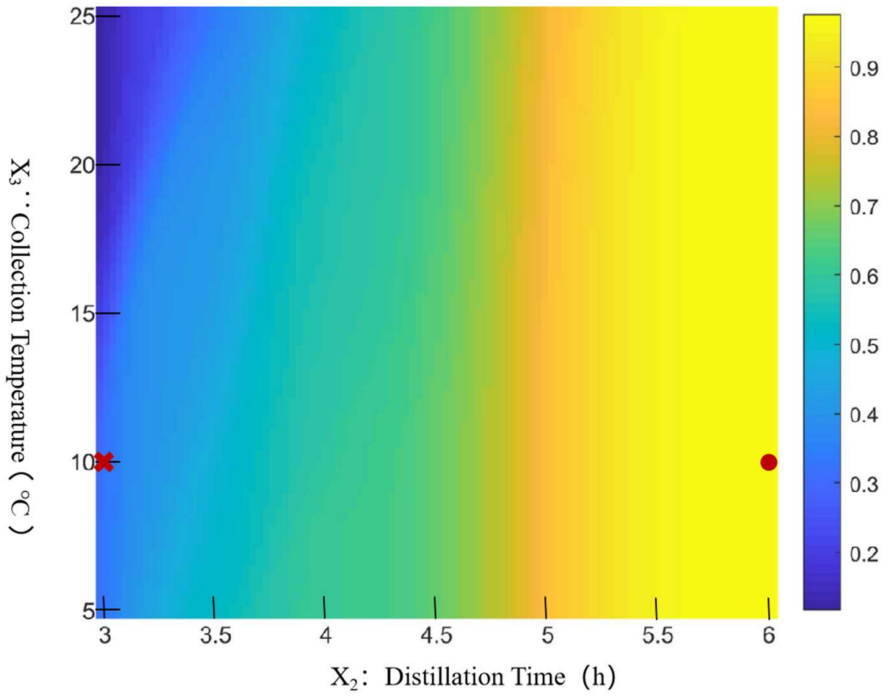


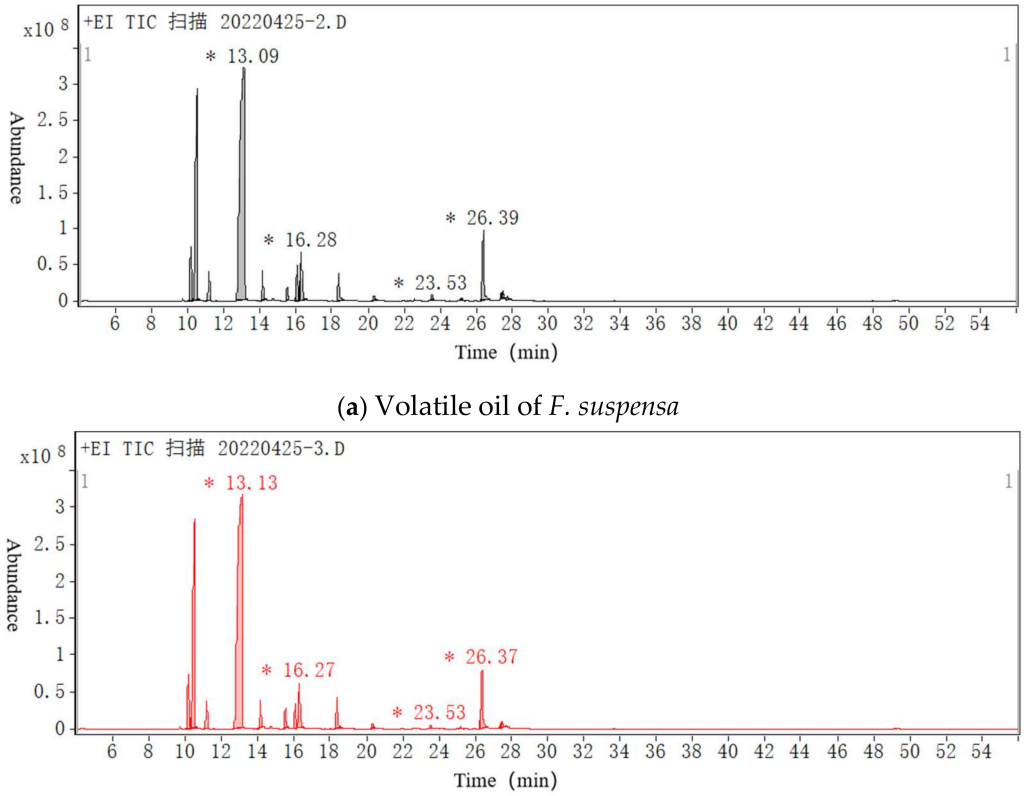
Figure 7. Two-dimensional design space diagram (water addition was fixed at 10 mL/g; "✖" is the verification point outside the design space; "●" is the verification point inside the design space; and the color bar on the right is the probability of meeting the standard).

3.5. Qualitative Analysis of Chemical Constituents of Volatile Oil

The distilled volatile oil of *F. suspensa* and the volatile oil of mixtures of *F. suspensa* and *L. japonica* were analyzed by GC–MS. The total ion chromatogram is shown in Figure 8, and the mass spectrometry results are shown in Tables 10 and 11. Interactions between multiple components can also have an impact on pharmacological activity [36,37]. A total of 16 chemical components were identified in the volatile oil of *F. suspensa*, among which the relative content of β-pinene was the largest (53.71%). Some other components with relatively large contents were α-pinene (21.12%), terpinene-4-ol (5.97%), (+)-limonene (4.36%), and α-thujene (4.25%). A total of 16 chemical components were identified in the volatile oil of *F. suspensa* and *L. japonica*, among which the relative content of β-pinene was also the largest (54.75%). Some other components with relatively large contents were α-pinene (21.38%), terpinene-4-ol (4.98%), (+)-limonene (4.48%), and α-thujene (4.41%).

Tables 10 and 11 show that the chemical composition of the volatile oil of *F. suspensa* was basically the same as that of the mixtures of *F. suspensa* and *L. japonica*. This meant that when *F. suspensa* and *L. japonica* were distilled together, the main contribution of the volatile oil chemical components came from *F. suspensa,* while *L. japonica* basically did not contribute. The combination of the two medicinal materials did not increase the amount of volatile oil distilled from *L. japonica*.

The authors attempted to distill the volatile oil from *L. japonica* but failed to collect any volatile oil. Li Jianjun et al. [20] studied the collection of volatile oil from *L. japonica* by steam distillation, and a total of 79 chemical components were identified in the volatile oil, among which the contents of palmitic acid (46.42%) and linoleic acid (14.32%) were the highest.



(**a**) Volatile oil of *F. suspensa*

(**b**) Volatile oil of *F. suspensa* and *L. japonica*

Figure 8. Total ion chromatogram of the GC–MS analysis of volatile oils.

Table 10. The volatile oil of *F. suspensa*.

No	Retention Time (min)	IUPAC Name	Common Name	Chemical Formula	Base Peak (*m*/*z*)	Relative Content (%)
1	10.14	Bicyclo [3.1.0]hex-2-ene, 2-methyl-5-(1-methylethyl)-	α-Thujene	$C_{10}H_{16}$	93.20	4.25
2	10.51	Bicyclo [3.1.1]hept-2-ene, 2,6,6-trimethyl-	α-Pinene	$C_{10}H_{16}$	93.29	21.12
3	11.15	Bicyclo [2.2.1]heptane, 2,2-dimethyl-3-methylene-	Camphene	$C_{10}H_{16}$	93.20	1.62
4	13.09	Bicyclo [3.1.1]heptane, 6,6-dimethyl-2-methylene-	β-Pinene	$C_{10}H_{16}$	93.29	53.71
5	14.13	1,6-Octadiene, 7-methyl-3-methylene-	β-Myrcene	$C_{10}H_{16}$	93.20	1.66
6	15.49	1,3-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-	α-Terpinene	$C_{10}H_{16}$	121.20	0.82
7	16.04	Benzene, 1-methyl-3-(1-methylethyl)-	m-cymene	$C_{10}H_{14}$	119.20	2.38
8	16.28	Cyclohexene, 1-methyl-4-(1-methylethenyl)-, (4R)-	(+)-Limonene	$C_{10}H_{16}$	93.20	4.36
9	18.35	1,4-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-	γ-Terpinene	$C_{10}H_{16}$	93.20	1.96
10	20.32	Cyclohexene, 1-methyl-4-(1-methylethylidene)-	Terpinolene	$C_{10}H_{16}$	93.20	0.38

Table 10. *Cont.*

No	Retention Time (min)	IUPAC Name	Common Name	Chemical Formula	Base Peak (*m*/*z*)	Relative Content (%)
11	23.53	Bicyclo [3.1.1]heptan-3-ol, 6,6-dimethyl-2-methylene-, (1S,3R,5S)-	(-)-trans-Pinocarveol	$C_{10}H_{16}O$	92.20	0.32
12	25.16	Bicyclo [3.1.1]heptan-3-one, 6,6-dimethyl-2-methylene-	Pinocarvone	$C_{10}H_{14}O$	81.20	0.18
13	26.39	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-	Terpinen-4-ol	$C_{10}H_{18}O$	71.20	5.97
14	27.36	3-Cyclohexene-1-methanol, α,α,4-trimethyl-	α-Terpineol	$C_{10}H_{18}O$	59.20	0.40
15	27.47	Bicyclo [3.1.1]hept-2-ene-2-carboxaldehyde, 6,6-dimethyl-	Myrtenal	$C_{10}H_{14}O$	79.20	0.50
16	27.69	Bicyclo [3.1.1]hept-2-ene-2-methanol, 6,6-dimethyl-	Myrtenol	$C_{10}H_{16}O$	79.20	0.39

Table 11. The volatile oil of mixtures of *F. suspensa* and *L. japonica*.

No	Retention Time (min)	IUPAC Name	Common Name	Chemical Formula	Base Peak (*m*/*z*)	Relative Content (%)
1	10.13	Bicyclo [3.1.0]hex-2-ene, 2-methyl-5-(1-methylethyl)-	α-Thujene	$C_{10}H_{16}$	93.20	4.48
2	10.50	Bicyclo [3.1.1]hept-2-ene, 2,6,6-trimethyl-	α-Pinene	$C_{10}H_{16}$	93.29	21.38
3	11.15	Bicyclo [2.2.1]heptane, 2,2-dimethyl-3-methylene-	Camphene	$C_{10}H_{16}$	93.20	1.63
4	13.13	Bicyclo [3.1.1]heptane, 6,6-dimethyl-2-methylene-	β-Pinene	$C_{10}H_{16}$	93.29	54.75
5	14.13	1,6-Octadiene, 7-methyl-3-methylene-	β-Myrcene	$C_{10}H_{16}$	93.20	1.56
6	15.49	1,3-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-	α-Terpinene	$C_{10}H_{16}$	121.20	1.23
7	16.03	Benzene, 1-methyl-3-(1-methylethyl)-	m-cymene	$C_{10}H_{14}$	119.20	1.66
8	16.27	Cyclohexene, 1-methyl-4-(1-methylethenyl)-, (4R)-	(+)-Limonene	$C_{10}H_{16}$	93.20	4.41
9	18.35	1,4-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-	γ-Terpinene	$C_{10}H_{16}$	93.20	2.29
10	20.32	Cyclohexene, 1-methyl-4-(1-methylethylidene)-	Terpinolene	$C_{10}H_{16}$	93.20	0.37
11	23.53	Bicyclo [3.1.1]heptan-3-ol, 6,6-dimethyl-2-methylene-, (1S,3R,5S)-	(-)-trans-Pinocarveol	$C_{10}H_{16}O$	92.20	0.28
12	25.17	Bicyclo [3.1.1]heptan-3-one, 6,6-dimethyl-2-methylene-	Pinocarvone	$C_{10}H_{14}O$	81.20	0.14
13	26.37	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-	Terpinen-4-ol	$C_{10}H_{18}O$	71.20	4.98
14	27.42	3-Cyclohexene-1-methanol, α,α,4-trimethyl-	α-Terpineol	$C_{10}H_{18}O$	59.20	0.28
15	27.47	Bicyclo [3.1.1]hept-2-ene-2-carboxaldehyde, 6,6-dimethyl-	Myrtenal	$C_{10}H_{14}O$	79.20	0.43
16	27.70	Bicyclo [3.1.1]hept-2-ene-2-methanol, 6,6-dimethyl-	Myrtenol	$C_{10}H_{16}O$	79.20	0.13

4. Conclusions

In this study, the steam distillation process of volatile oil from *F. suspensa* and *L. japonica* was optimized according to the concept of quality by design. First, the liquid/material ratio, distillation time, and collection temperature were identified as critical process parameters by a search of the literature and single-factor experiments. In addition, this study further investigated the effect of different batches of *F. suspensa* on the distillation of volatile oil and determined that the critical raw material properties were the volatile oil content and β-pinene content. HPLC was used to evaluate the different batches. The content of β-pinene in *F. suspensa* was measured, and related methodological verification work was performed. Then, a Box–Behnken design was used to study the quantitative relationship between three process parameters, two raw material properties, and the volatile oil yield. A mathematical model was established with R^2 exceeding 0.90. Furthermore, the design space of the volatile yield was calculated by a probability-based method, and verification experiments were carried out. The measured values of two verification points were relatively close to the model-predicted values, indicating that the mathematical model established according to the Box–Behnken design was accurate. The volatile oil yield at the verification point in the design space was greater than the preset standard, and the volatile oil yield at the verification point outside the design space was lower than the preset standard, indicating that the design space was reliable. In this study, the chemical constituents of the volatile oil of *F. suspensa* and the volatile oil of *F. suspensa* and *L. japonica* were analyzed by GC–MS. A total of 16 chemical constituents were identified in the volatile oil of *F. suspensa*, among which the content of β-pinene was the largest (53.71%). A total of 16 chemical constituents were identified in the volatile oil of *F. suspensa* and *L. japonica*, among which the content of β-pinene was also the largest (54.75%), and the composition was similar to that of the volatile oil of *F. suspensa*. The results showed that when *F. suspensa* and *L. japonica* were distilled together, the main contributor to the volatile oil was *F. suspensa*. The contribution of *L. japonica* was small. The combination of *F. suspensa* and *L. japonica* did not increase the volatile oil yield from *L. japonica*.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/separations10010025/s1, Table S1: Sample Recovery Test Results.

Author Contributions: Conceptualization, X.G., N.W. and Z.W.; investigation, X.C. and D.G.; data curation, X.C. and X.G.; writing—original draft preparation, X.C., X.G. and N.W.; writing—review and editing, X.G., Z.W. and N.W.; supervision, N.W. and X.G.; funding acquisition, X.G., N.W. and Z.W. All authors have read and agreed to the published version of the manuscript.

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