Ultrasonic-assisted extraction and adsorption separation: Large-scale preparation of trans-\(\epsilon\)-Viniferin, suffruticosol B and trans-Gnetin H for the first time

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Abstract

In this study, a Standard Operating Procedure (SOP) for the large-scale extraction, enrichment, and separation of suffruticosol B (SB), trans-\(\epsilon\)-Viniferin (TV), trans-Gnetin H (TG) from oil tree peony seeds shell (PSS) was successfully constructed. The ultrasonic-assisted extraction (UAE), macroporous adsorption resin (MAR), and column chromatography (CC) were employed to extract, enrich and separate SB, TV and TG from PSS, and the conditions were optimized. The results implied that SB (1.6937 g), TV (0.5884 g) and TG (3.8786 g) with the purity of 99.67 %, 99.32 % and 98.54 %, respectively, were obtained after the extraction, enrichment and separation. The total yields of the SB, TV and TG were 0.61 mg/g, 0.02 mg/g and 6.64 mg/g with the total extraction rates at 70.55 %, 69.77 % and 78.36 %, respectively. This is the first report on the large-scale extraction, enrichment and separation of oligostilbenes. The SOP in this paper could produce high purity SB, TV and TG, and provide a new idea for PSS as a new oligostilbene resource. The study expands the new development and research field of PSS and provides theoretical support for the green utilization of oil tree peony.

1. Introduction

Oligostilbenes are natural products with novel skeleton structure formed by the polymerization of resveratrol and its derivatives in different ways \([1]\). Studies revealed that oligostilbenes were mainly distributed in families of Dipterocarpaceae, Vitiaceae, Cyperaceae, Leguminosae and Magnoliaceae, and there was also a small amount of oligostilbenes found in families of Iridaceae, Celastraceae, Paonioniaceae and Moraceae \([1,2]\). So far, only about 500 oligostilbenes had been isolated from nature \([2]\). At present, there are no reports on the large-scale extraction, enrichment and separation of these compounds owing to the small distribution and relatively low content of oligostilbenes in nature. Therefore, the study has a good guiding role in the research and development of oligostilbenes.

It was found that oligostilbenes existed in the seeds, mainly concentrated in the seeds shell of species of genus Paeonia \([3-6]\). The research of our research group found that the seeds shell of oil tree peony contained a variety of oligostilbenes, and these compounds were presented at high levels in the seeds shell of oil tree peony. Among them, SB, TV and TG had the highest content with important development and utilization value \([3,7,8]\). The bioactivities evaluation revealed that SB, TV and TG had good antioxidant \([5]\), antibacterial \([9]\), anti-inflammatory \([10,11]\), anti-tumor \([4,9,12]\), cognitive function improvement \([13]\), hypoglycemic effects \([4]\), enzyme inventory activity \([4]\), etc. As a kind of authorized edible oil, tree peony seed oil (TPSO) was widely concerned for its rich content of unsaturated fatty acids, which led to the increasing cultivation area of oil tree peony year by year \([14,15]\). As a principal byproduct in TPSO production process, PSS occupied over 30 % of the total mass of oil tree peony seeds \([3,8,9,15]\). Therefore, the study on the extraction, enrichment and separation of oligostilbenes from PSS has broad development and application space.

The molecular structures of oligostilbenes in PSS possessed more...
phenolic hydroxyl groups, which resulted in the relative instability of oligostilbenes [3,5,9]. UAE was an effective method to extract oligostilbenes for the low extraction temperature, short extraction time and high efficiency [7,9,16,17]. The primary goal of this research is to design a fast and efficient Standard Operating Procedure (SOP) for obtaining SB, TV and TG from PSS. Therefore, on the basis of previous studies, UAE with ethanol was used to extract SB, TV and TG from PSS, on the basis of further enrichment by MAR, silica gel CC and Sephadex LH-20 CC were employed to isolate SB, TV and TG. This SOP will help to increase add-on value of oil tree peony deep-processing products and will serve as a research reference for the in-depth development of bioactive chemicals SB, TV and TG.

2. Materials and methods

2.1. Materials

The PSS, provided by Henan Housheng Biotechnology Co., ltd., was washed, dried and crushed (100 mesh), and then was kept at -4 °C for the following use. The ethanol, methanol, dichloromethane and acetonitrile were bought from Luoyang Chongtian Chemical Glass Co., Ltd. All chemical reagents are analytical grade or HPLC grade.

Beijing Ruida Henghui Technology Development Co., Ltd. produced the MAR HPD417 and HPD100, HZ806 and D101 were obtained from Shanghai Huazhen Technology Co., Ltd., and HZ816 were supplied by Zhengzhou Hecheng New Material Technology Co., Ltd..

The standards of SB, TV and TG were obtained from PSS by our research group, and the structures and purity of each reference was determined by spectral and chromatographic methods [3].

2.2. Determination of SB, TV and TG by HPLC-DAD

An Agilent 1100 HPLC series system (Agilent, USA) was employed for the content determination of SB, TV and TG, and the chromatographic conditions were optimized on the basis of literature with a slightly adjustment [3].

Compounds SB, TV and TG in the samples were confirmed by comparing with the retention time of the references. The standard working curve created by an external standard method was used to determine the content of SB, TV and TG in the sample [3]. The study revealed that SB, TV and TG had good linearity in the range of 19.6–313.6 μg/mL, 20.0–100.0 μg/mL, and 19.4–310.4 μg/mL, respectively. The regression curves of SB, TV and TG were \( y = 5.9186x - 199.82 \) (\( r^2 = 0.9990 \)), \( y = 26.107x - 213.45 \) (\( r^2 = 0.9994 \)) and \( y = 7.4797x + 415.55 \) (\( r^2 = 0.9996 \)), respectively, where \( x \) was the concentration of the standard sample (μg/mL) and \( y \) was the peak area.

2.3. Extraction of SB, TV and TG

The effects of liquid to solid ratio (10:1, 15:1, 20:1, 25:1, 30:1), extraction time (10, 20, 30, 40, 50 min), extraction temperature (15, 25, 35, 45, 55 °C) and ethanol concentration (50, 60, 70, 80, 90 %) on the yields of SB, TV and TG were investigated using an ultrasonic extractor (Fig. 1). To make a solution with a specific concentration, the filtrate extracted was concentrated under reduced pressure. The yield was calculated using the following formula after the contents of SB, TV and TG were determined using the standard HPLC method.

\[
Y = \frac{C_0 \times V \times 1000}{M}
\]

Where \( Y \) is yield (mg/g); \( V \) is the test solution volume (mL); \( C_0 \) is the test solution concentration (μg/mL); \( M \) is the mass of extract (g).

2.4. RSM design and statistical analysis of the UAE

The time (\( X_1 \)), temperature (\( X_2 \)) and ethanol concentrations (\( X_3 \)) of the extraction were chosen for the RSM analysis of the three factors and three levels Box-Behnken design to determine the best extraction conditions.
process based on the research results of the single-factor experiment. Table S1 showed the experimental settings and outcomes, whereas Fig. 2 suggested the reaction results. The data was analyzed using the Design-Expert application. Analysis of variance (ANOVA) was used to assess the accuracy of the model and the relevance of process parameters, and the findings were displayed in Table 1.

The response value of the optimal extraction procedure was calculated using the second-order polynomial model:

$$Y = a_0 + \sum_{i=1}^{n} a_i x_i + \sum_{i=1}^{n} a_{ii} x_i^2 + \sum_{i=1}^{n} \sum_{j=i+1}^{n} a_{ij} x_i x_j$$  \hspace{1cm} (2)

Where $Y$ is the response variable (mg/g); $a_0$ is a constant; $a_i$, $a_{ii}$ and $a_{ij}$ are linear effect, square effect and mutual effect, respectively; $x_i$ and $x_j$ are the independent variables.

2.5. Adsorption isotherm experiments of SB, TV and TG

The static adsorption and desorption of HPD417, HPD100, HZ806, D101 and HZ816 were evaluated and the adsorption and desorption rates of the five resins were compared (Table 2). According to the results of adsorption and desorption, a suitable resin was selected to enrich the oligostilbenes of SB, TV and TG.

The PSS crude extract solution (20 mL) with different initial concentrations (0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4 mg/mL) was added into the screened MAR (HZ816) (5 g), respectively, shook for 12 h at 25 °C. The contents of SB, TV and TG were measured in each solution before and after adsorption for the determination of the adsorption capacity versus time curve (Fig. 3(a)). And the adaptability of SB, TV and TG to the Langmuir model and Freundlich model was evaluated.

Langmuir equation describes ideal single-molecule adsorption, and...
ANOVA results and significance of RSM model.

| Source | Sum of Squares | Df | Mean Square | F-value | p-value (Significant) | p-value (Significant) | p-value (Significant) |
|--------|----------------|----|-------------|---------|-----------------------|-----------------------|-----------------------|
| SB     | 99.85          | 6  | 16.64       | 29.82   | 0.0001(*)             | 0.0001(*)             | 0.0001(*)             |
| TV     | 10.43          | 6  | 1.74        | 3.28    | 0.0458                | 0.0458                | 0.0458                |
| TG     | 24.05          | 6  | 4.00        | 7.59    | 0.0001(**)            | 0.0001(**)            | 0.0001(**)            |

 Suffrutosol B(B): R² = 0.9992; adjusted R² = 0.9997; coefficient of variation(cv) = 0.1231.

 Viniferin(V): R² = 0.9950; adjusted R² = 0.9993; coefficient of variation(cv) = 0.4097; PRESS = 0.0303.

 Trans-Gnetin H(H): R² = 0.9922; predicted R² = 0.9694; adeq precision = 0.2578; PRESS = 0.5271.

its model can be expressed by the following formula:

\[ Q_e = \frac{Q_{max}K_fC_s}{1 + K_fC_s} \]  

(3)

Where \( C_s \) is the solution concentration after adsorption equilibrium (mg/mL); \( Q_e \) is the adsorption capacity of the resin at adsorption equilibrium (mg/g); \( K_f \) is Langmuir coefficient (mg/mL); \( Q_{max} \) is the maximum adsorption capacity of the resin (mg/g).

Freundlich equation is applicable to non-ideal heterogeneous adsorption, and its model can be expressed as:

\[ Q_e = K_fC_s^{1/n} \]  

(4)

Where \( K_f \) is the Freundlich constant; \( 1/n \) is an empirical constant related to the size of the driving force for adsorption.

2.6. Static adsorption and desorption kinetics of SB, TV and TG

The curves of adsorption capacity (\( Q_e \)), desorption capacity (\( Q_{des} \)) and adsorption time (\( t \)) of the enrichment of HZ816 for SB, TV and TG at 25 °C were shown in Fig. 3(b-c). Pseudo-first-order model and Pseudo-second-order model are classical models for studying adsorption kinetics. The two models can be expressed as follows:

\[ \ln(Q_e - Q_t) = \ln(Q_e - \frac{Q_{des}}{K_f}) + \frac{K_f}{Q_e}t \]  

(5)

\[ \frac{t}{Q_e} = \frac{1}{K_fQ_e} + \frac{t}{Q_{des}} \]  

(6)

Where \( Q_e \) and \( Q_{des} \) represent the adsorption capacity at equilibrium and at a certain time \( t \), respectively (mg/g); \( K_f \) is the rate constant of pseudo-first-order reaction (h\(^{-1}\)); \( K_2 \) is the rate constant of pseudo-second-order reaction (g/(mg h)); \( T \) is the adsorption time (h).

2.7. Dynamic adsorption and elution of SB, TV and TG

MAR HZ816 (15 g) was put into a glass column (1.6 × 36 cm) by wet method. The optimal enrichment process was determined by comparing different sample loading flow rates (1, 2, 3, 4, 5 BV/h), different eluent dosage (2, 3, 4, 5, 6, 7, 8, 9 BV) and different eluent ethanol concentration (0, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 %) on the results of enrichment (Fig. 4).

The enriched sample was concentrated under vacuum, dissolved, and then fixed to an equal volume. The solution was filtered, and then was determined by HPLC, the contents of SB, TV and TG, along with the adsorption and desorption rates of the compounds were calculated.

\[ Q_e = \frac{V_1(C_s - C_e)}{W} \]  

(7)

\[ \eta = \frac{(C_s - C_e)V_1}{C_sC_0} \times 100 \]  

(8)

\[ D = \frac{C_sV_2}{(C_s - C_e)\times 100} \]  

(9)

Where \( Q_e \) is the adsorption capacity of the resin at adsorption equilibrium (mg/g); \( \eta \) is the adsorption rate (%); \( D \) is desorption rate (%); \( W \) is the mass of dry resin (g); \( C_s \) is the mother liquor concentration (mg/mL); \( C_e \) is the desorption solution concentration (mg/mL); \( C_0 \) is the solution concentration after adsorption equilibrium (mg/mL); \( V_1 \) is the adsorption solution volume (mL); \( V_2 \) is the desorption solution volume (mL).

2.8. The isolation of SB, TV and TG

The enriched sample (8.7169 g) was dissolved in methanol, and then 13 g silica gel was added, and mixed evenly. After volatilizing solvent, the mixture was ground, and chromatographed over a silica gel CC (10
× 160 cm, 300–400 mesh, 2000 g), and eluted with a gradient of the dichloromethane-methanol system (15:1, 12:1, 10:1, V/V), and then collected each fraction. The contents of SB, TV and TG in each fraction were determined by the HPLC method [3], and similar fractions were combined according to the analysis results, and then three fractions (Fr. 1–3) were obtained.

The three fractions (Fr. 1–3) were dissolved in methanol, and then chromatographed over a Sephadex LH-20 CC (10 × 160 cm, 500 g), respectively, and eluted with methanol. The subfractions of SB, TV and TG were collected based on the analysis results of the HPLC method [3].

3. Results and discussion

3.1. HPLC method validation

HPLC was used to measure the amounts of SB, TV and TG in the samples, and the method was tested for precision, stability, repeatability and sample recovery (Table 3). This approach had good precision, accuracy and stability (Table 3), and was suitable for the analysis of SB, TV and TG in samples.

Table 2

| Type     | SB Adsorption rate (%) | Desorption rate (%) | TV Adsorption rate (%) | Desorption rate (%) | TG Adsorption rate (%) | Desorption rate (%) |
|----------|------------------------|---------------------|------------------------|---------------------|------------------------|---------------------|
| HPD100   | 79.96                  | 82.14               | 76.91                  | 76.57               | 85.13                  | 84.15               |
| D101     | 69.32                  | 71.88               | 71.23                  | 71.32               | 82.24                  | 80.72               |
| HPD417   | 77.29                  | 76.59               | 74.39                  | 74.42               | 81.01                  | 82.59               |
| HZ801    | 67.69                  | 72.39               | 68.38                  | 61.95               | 74.67                  | 71.91               |
| HZ816    | 82.94                  | 84.99               | 80.24                  | 88.06               | 86.33                  | 88.53               |

3.2. Single-factor experiments

The effects of liquid-to-solid ratio, ultrasonic time, ultrasonic temperature, and ethanol concentration on the yields of SB, TV and TG were evaluated (Fig. 1). The results suggested that the influence trend of liquid-to-solid ratio, ultrasonic time, ultrasonic temperature and ethanol concentration on the yield of SB, TV and TG was basically the same. With the increase of the level of each factor, the yields of SB, TV and TG first increased and then decreased, and the maximum values appeared at 35°C, 15 min, 15 mL/g and 70% ethanol, respectively. When the liquid-to-solid ratio was 10–15 mL/g, the yield also increased with the increase of the liquid-to-solid ratio, and the maximum yield was achieved at 15 mL/g. The increase in solvent volume would lead to a decrease in mass transfer efficiency and dissolution rate [18]. Therefore, in order to avoid the waste of solvent, the optimal liquid-to-solid ratio was set up at 15 mL/g in following experiments. The levels of ultrasonic temperature (25, 35, 45°C), ultrasonic time (10, 15, 20 min), and ethanol...
Designed by Design Expert 12, the RSM was employed to evaluate the levels were selected (Table 3). Through 17 groups of experiments by macroporous resin.

3.3. The RSM design and statistical analysis

On the basis of single factor experiments, three factors and three levels were selected (Table S1). Through 17 groups of experiments designed by Design Expert 12, the RSM was employed to evaluate the effects of ultrasonic temperature, ultrasonic time, and ethanol concentration on the yield of SB, TV, and TG by UAE. The results were presented in Table S1.

The following second-order polynomial models were used to calculate the response values of the optimal extraction process of SB (Y1), TV (Y2) and TG (Y3), respectively.

\[
Y_1 = 31.14 + 0.1750X_1 + 0.2763X_2 + 0.4588X_3 - 0.2775X_1X_2 + 0.0225X_1X_3 - 0.4050X_2X_3 - 1.20X_1^2 - 1.11X_2^2 - 1.30X_3^2
\]

(10)

\[
Y_2 = 5.70 + 0.0875X_1 + 0.0575X_2 + 0.1625X_3 + 0.0900X_1X_2 - 0.0300X_1X_3 + 0.0500X_2X_3 - 0.4485X_1^2 - 0.2685X_2^2 - 0.2585X_3^2
\]

(11)

\[
Y_3 = 62.70 + 0.4388X_1 + 0.3063X_2 + 0.5800X_3 - 0.03125X_1X_2 + 0.0900X_1X_3 + 0.3750X_2X_3 - 2.00X_1^2 - 1.43X_2^2 - 1.74X_3^2
\]

In accordance with the results of analysis of variance analysis (ANOVA) of the model (Table 1), the P values of the models (P < 0.0001) were very significant, and the P values of lack of fit (P = 0.6032, 0.3271 and 0.3613, respectively) were not significant. Which implied that the regression models could be well fitted with the actual measured values [19,20] and could correctly reflect the relationship between the three variables (extraction time (X1), extraction temperature (X2) and ethanol concentration (X3)) and the yields of SB, TV and TG by UAE. In addition, the coefficient of variation (CV) of the models were 0.1767 %, 0.4097 % and 0.2578 %, respectively, indicating that the authenticity and reproducibility of the developed model were very high [21].

The determination coefficients (R²) (0.9992, 0.9983 and 0.9966, respectively) of the three models were all near to 1, implying that the model could match the experimental results well. As a result, the fitting model was enough to characterize the experimental data and might be used to forecast the actual yield of SB, TV and TG. For the adjusted R² (adj R²) (0.9982, 0.9962 and 0.9922, respectively) and the predicted coefficient of determination (pred-R²) (0.9949, 0.9843 and 0.9694, respectively), the difference is much <0.2 [22,23]. The results implied that the predicted value was similar to the experimental value. The high pred-R² also confirmed the significance and good regression performance of the models [21]. Therefore, the fitting models were sufficient to represent the experimental data and could be employed to calculate the actual yields of SB, TV and TG.

The interaction among three independent variables (X1, X2 and X3), quadratic terms (X1², X2² and X3²), extraction time (X1) and extraction temperature (X2), extraction temperature (X2) and ethanol concentration (X3) of SB, TV and TG were very significant (P < 0.01). The interaction of extraction time (X1) and ethanol concentration (X3) of TV and TG had a significant effect on the yields (0.01 < P < 0.05). The value of F was positively correlated with the effects of three factors on the yields of SB, TV and TG [24]. On the basis of the F-value of the models, the factors influencing the yields of SB and TV were: ethanol concentration (X3) > extraction temperature (X2) > extraction time (X1); while the yield of TG was: ethanol concentration (X3) > extraction time (X1) > extraction temperature (X2). The results indicated that the main factor which affecting the yields of SB, TV and TG was ethanol concentration.

![Fig. 4. Effects of flow rate, eluent dosage, and ethanol concentration on purification of SB, TV, and TG by macroporous resin.](image-url)
3.4. Analysis of response surface

The yields of SB, TV and TG were affected by the extraction time ($X_1$) and extraction temperature ($X_2$) when the ethanol concentration ($X_3$) was fixed (Fig. 2 (A, H, N)). The yields of SB, TV and TG increased as the extraction period increased from 10 to 15 min, and the yields increased slowly after 15 min. Furthermore, as the temperature rose from 25 to 35 °C, the yields of SB, TV and TG increased, then fell slightly. The main reason for the increase of the yields with the increase of time and temperature was that during UAE, the ultrasonic wave was transformed into heat energy after passing through the cell wall, causing the temperature around the cell wall to rise and burst, and the active components in the cell were dissolved to achieve the extraction effect [23]. However, with the further increase in temperature, solvent volatilization led to the decrease of the solvent, resulting in a decrease in the extraction rate. This was also observed by the yields of SB, TV and TG affected by the extraction temperature ($X_2$) and ethanol concentration ($X_3$) when extraction time ($X_1$) was fixed (Fig. 2 (E, L, R)). As the extraction temperature gradually increased, the yields of SB, TV and TG increased gradually, but when the temperature exceeded 35 °C, with the increase of temperature and the conversion of the ultrasonic wave into heat energy, the solvent volatilized, and the increase of the yields of SB, TV and TG was not obvious. The yields of SB, TV and TG increased dramatically with the ethanol concentration increased from 60 % to 70 %, and then decreased slightly when the ethanol concentration was over 70 %.

According to the analysis of the models established by RSM, the optimal prediction conditions for SB were 15.32 min, 35.87 °C and 71.64 % ethanol; for TV were 15.31 min, 36.52 °C and 73.85 % ethanol; and for TG were 15.48 min, 36.19 °C and 71.17 % ethanol. The corresponding optimum yields of SB, TV and TG were 31.20 mg/g, 5.74 mg/g and 62.16 mg/g, respectively. In order to facilitate the experiment, the corresponding optimum yields of SB, TV and TG were determined by HPLC (Fig. 5 (c)). As the volume of desorption solution reaches 4 BV, SB, TV and TG could be almost completely desorbed, so the optimal volume of desorption solution was determined to be 4 BV. The desorption rate of the three compounds was the highest at 70 % ethanol (Fig. 4(c)). As the concentration of ethanol increased, the desorption rate was almost unchanged, so 70 % ethanol was selected for desorption.

3.5. Static adsorption isotherms

Generally, the adsorption of the monolayer is described by the Langmuir model, HZ816 MAR is a non-polar adsorbent, the surface of the non-polar adsorbent is uniform, and the adsorption is monolayer [25,26]. This conclusion was supported by the results (Table 4). The model parameters and correlation coefficient ($R^2$) (Table 4) revealed that the Langmuir model had high $R^2$, indicating that the Langmuir model was more suitable to interpret the adsorption of HZ816 on SB, TV and TG. Generally, the Langmuir constant ($K_L$) value of the Langmuir model implies whether the isotherm is favorable (0 < $K_L$ < 1), linear ($K_L$ = 1), or unfavorable ($K_L$ greater than 1) [27]. The results in Table 4 revealed that the $K_L$ values of SB, TV and TG were <1, indicating that the adsorption of HZ816 on SB, TV and TG conformed to the Langmuir model. In the Freundlich model, the adsorption strength value ($1/n$) of 0.1 < $1/n$ < 0.5, 0.5 < $1/n$ < 1, and greater than 1 means the adsorption is easy to occur, hard to adsorb, and almost no adsorption, respectively [28]. The results in Table 4 suggested that the values of $1/n$ of SB, TV and TG were all greater than 1, implying that the adsorption of SB, TV and TG on HZ816 resin did not conform to the Freundlich model.

3.6. Static adsorption and desorption kinetics

Fig. 3(b) suggested that the adsorption process of SB, TV and TG was divided into three stages [29,30]. In the first stage, the adsorption rate increased with great speed in the first 5 h and then increased tardily. After 12 h, the steady-state approximation appeared. In the industrial production of natural pharmaceutical chemistry, it is very important to determine the contact time of the adsorbent for optimizing industrial production [25].

The simulation results of adsorption kinetics of HZ816 MAR for SB, TV and TG were shown in Table 5. The results revealed that the adsorption kinetics of the MAR for SB, TV and TG conformed to the pseudo-first-order model, and the $Q_e$ calculated by the pseudo-first-order model was closer to the actual $Q_e$. The results in Table 4 suggested that the values of $1/n$ of SB, TV and TG on HZ816, and the results implied that the desorption equilibrium was reached after 6 h. Combined with the actual industrial production, the time of static adsorption and desorption was 12 h and 6 h, respectively.

3.7. Optimization of enrichment process of SA, TV and TG

It was found that the adsorption rates of SB, TV and TG decreased with the increase of sample loading flow rate, the adsorption effect was the best when the sample loading flow rate was 2 BV/h. So the sample loading flow rate was determined as 2 BV/h (Fig. 4(a)). The desorption rates of SA, TV and TG gradually increased with the increase of desorption solution volume (Fig. 4(c)). When the volume of desorption solution reaches 4 BV, SB, TV and TG could be almost completely desorbed, so the optimal volume of desorption solution was determined to be 4 BV. The desorption rate of the three compounds was the highest at 70 % ethanol (Fig. 4(c)). As the concentration of ethanol increased, the desorption rate was almost unchanged, so 70 % ethanol was selected for desorption.

Based on the best conditions obtained from the above results, the extraction sample (20 g) was enriched at HZ816 with the initial sample concentration of 0.8 mg/mL, and loaded 2 BV at 2 BV/h loading flow rate, and removed the impurities with purified water, and then desorbed with 4 BV 70 % ethanol. The desorption solution was collected, concentrated under reduced pressure, and then the contents of SB, TV and TG were determined by HPLC (Fig. 5(c)).

After enrichment with HZ816, a total of product (8.7169 g) was obtained, and the contents of SB, TV and TG increased from 109.23 mg/g, 38.53 mg/g and 235.64 mg/g to 228.64 mg/g, 79.42 mg/g and 494.41 mg/g, respectively. The yields of SB, TV and TG were 111.09 mg/g, 44.01 mg/g and 240.20 mg/g after enrichment, respectively, and the extraction rates were 91.23 %, 89.86 % and 91.54 %, respectively. The above results implied that the enrichment process conditions and parameters were stable, feasible and repeatable, and were suitable for industrial production [27,28].

| Sample name  | Langmuir equation | $R^2$ | Freundlich equation | $R^2$ | $1/n$ |
|--------------|-------------------|-------|---------------------|-------|-------|
| Saffronisol B| $Q = 30.1805C + 12.6007$ | 0.9556 | $Q = 8.7328C^{0.4502}$ | 0.8820 | 0.4502 |
| Trans- e-Viniferin | $Q = 20.8058C + 5.7357$ | 0.9754 | $Q = 4.4397C^{0.3706}$ | 0.8896 | 0.3706 |
| Trans-Gnetin H | $Q = 39.3437C + 14.4742$ | 0.9557 | $Q = 10.4053C^{0.4265}$ | 0.8697 | 0.4265 |
3.8. The isolation of SB, TV and TG

The enriched sample (8.7169 g) was separated on silica gel CC (10 × 160 cm, 300–400 mesh, 2000 g), and three fractions (Fr. 1–3) were obtained. Fraction 1 (0.5195 g) was collected in 3500–5700 mL eluent (CH$_2$Cl$_2$: MeOH = 15:1), fraction 2 (1.7657 g) was collected in 5700–9900 mL eluent (CH$_2$Cl$_2$: MeOH = 12:1), fraction 3 (4.0558 g) was collected in 9900–14200 mL eluent (CH$_2$Cl$_2$: MeOH = 10:1). The HPLC detection results revealed that the main components of fractions 1–3 were TV, TG and SB, respectively.

The samples of three fractions (Fr.1–3) were dissolved in methanol, and then chromatographed on a Sephadex LH-20 CC (10 × 160 cm, 500 g) with methanol was used as the eluent, respectively. The subfractions of SB, TV and TG were collected based on the analysis results of the HPLC method. The relationship between elution volume and concentration of SB, TV and TG during purification was shown in Fig. 6. After purification, SB (1.5891 g), TV (0.5195 g) and TG (3.7313 g) were obtained with the purity of 99.67 %, 98.54 % and 99.32 %, respectively (Fig. 5(d–f)).

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After separation by silica gel CC and purification by Sephadex LH-20 CC, the yields of SB, TV and TG were 182.30 mg/g, 59.60 mg/g and 428.05 mg/g, respectively, and the extraction rates were 79.73 %, 75.04 % and 86.58 %, respectively, and the purity was all above 98 %. Which implied that the separation process was suitable for the separation of SB, TV and TG.

4. Conclusions

Large-scale preparation of natural active ingredients with high purity is the key to the development of natural products. In this study, a SOP for the large-scale extraction, enrichment, and separation of SB, TV and TG from PSS was successfully constructed. The results showed that the optimum conditions for UAE were the extraction time of 15 min, the extraction temperature of 36 °C, and the ethanol concentration of 72 % with the liquid–solid ratio of 15 mL/g. Under the optimum conditions, the yields of SB, TV and TG were 31.10 ± 0.25 mg/g, 5.69 ± 0.13 mg/g and 62.15 ± 0.18 mg/g, respectively. The extracted samples were enriched by HZ816, and the optimum conditions were obtained: the sample concentration was 0.8 mg/mL with the loading flow rate of 2 BV/h, the ethanol concentration of the desorption solution was 70 %, and the ethanol dosage of desorption solution was 4 BV. After enrichment, the contents of SB, TV and TG increased from 109.32 mg/g, 38.53 mg/g and 235.64 mg/g to 228.64 mg/g, 79.42 mg/g and 494.41 mg/g, respectively. The enriched samples were separated by silica gel CC and purified by Sephadex LH-20 CC to obtain SB (1.6937 g), TV (0.5884 g) and TG (3.8786 g) with the purity of 99.67 %, 99.32 % and 98.54 %, respectively.

By UAE, MAR enrichment, silica gel CC and Sephadex LH-20 CC separation and purification, the total yields of the SB, TV and TG were 0.61 mg/g, 0.02 mg/g, and 6.64 mg/g, respectively; the total extraction rates were 70.55 %, 69.77 % and 78.36 %, respectively. This was the first report on the large-scale extraction, enrichment and separation of oligostilbenes. The results of pharmacological studies revealed that SB, TV and TG had good antioxidant [5], antibacterial [9], anti-inflammatory [10,11], anti-tumor [4,9,12], cognitive function improvement [13], hypoglycemic effects [4], enzyme inventory activity [4], etc. The SOP in this paper could produce high purity SB, TV and TG, and provided theoretical support for the green development of oil tree peony.

Table 5
Pseudo-first-order and Pseudo-second-order kinetic parameters of adsorption of SB, TV and TG on HZ816 resin.

| Type             | $Q_e$ (mg/g) | Pseudo-first-order parameters | Pseudo-second-order parameters |
|------------------|--------------|-------------------------------|-------------------------------|
|                  |              | $Q_1^f$ (mg/g) | $K_1^f$ (h$^{-1}$) | $R^2$ | $Q_2^f$ (mg/g) | $K_2$ (g/(mg*h)) | $R^2$ |
| Suffruticosol B  | 7.03         | 7.31                  | 0.2492                   | 0.9950 | 9.31         | 0.0261                  | 0.9832 |
| Trans-ε-Viniferin| 3.55         | 3.61                  | 0.2635                   | 0.9973 | 4.55         | 0.0582                  | 0.9883 |
| Trans-Gnetin H   | 9.28         | 9.77                  | 0.2266                   | 0.9963 | 12.64        | 0.0167                  | 0.9888 |

Fig. 5. HPLC profiles: (a) mixed reference substance of SB, TV and TG, (b) extracted sample, (c) enriched sample, (d) separated SB, (E) separated TV, (F) separated TG.

Fig. 6. The relationship between the number of tubes and the concentration during purification.
Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data that has been used is confidential.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ultsonch.2022.106123.

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