Screening study of blood-supplementing active components in water decoction of *Angelica sinensis* processed with yellow rice wine based on response surface methodology

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**ABSTRACT**

**Context:** *Angelica sinensis* (Oliv.) Diels (Apiaceae) (syn. *Angelica polymorpha* Maxim var. *sinensis* Oliver) processed with yellow rice wine (WAS) has a blood-supplementing effect.

**Objective:** To establish an optimal technology for preparing water decoction of WAS (WASD), and screen blood-supplementing fractions.

**Materials and methods:** Ferulic acid and crude polysaccharide were used in optimizing the preparation technology for WASD through response surface methodology. The independent variables were liquid–solid ratio, soaking time, and extraction time. Eighty Kunming mice were randomly divided into normal control, model, and six intervention groups (*n* = 10). The intervention groups were given different WASD fractions by gavage (5 or 10 g/kg). The model intervention groups received acetylphenyl hydrazine (subcutaneous injection) and cyclophosphamide (intraperitoneal injection). Duration of study, 9 days. The components of blood-supplementing fractions were analyzed.

**Results:** The optimum extraction parameters were liquid–solid ratio, 7.69:1 mL/g; soaking time, 119.78 min; and extraction time, 143.35 min. The optimal OD value was 0.8437. RBC, WBC, and Hb in the water fraction (5, 10 g/kg) and n-butanol fraction (10 g/kg) intervention groups increased significantly compared with the model group (*p* < 0.05). Polysaccharide and caffeic acid contents of water fraction were 252.565 and 0.346 μg/mg, respectively; ferulic acid was not detected. Caffeic acid and ferulic acid contents of *n*-butanol fraction were 1.187 and 0.806 μg/mg, respectively, polysaccharide was not detected.

**Conclusions:** The optimum preparation technology of WASD was obtained, and the water, *n*-butanol fractions were blood-supplementing fractions. This study provides a theoretical foundation for further application of WAS in the pharmaceutical industry.

**Introduction**

Blood deficiency syndrome (BDS) is one of the basic types of traditional Chinese medicine (TCM) syndromes. BDS can be seen in many diseases (Huo et al. 2010) and is widely harmful; thus, the enhancement of immunity and enrichment of blood is essential. Immunity considerably decreases when BDS occurs. Therefore, exploiting blood-supplementing drugs is of great significance.

*Angelica sinensis* (AS), the roots of *Angelica sinensis* (Oliv.) Diels (Apiaceae) (syn. *Angelica polymorpha* Maxim var. *sinensis* Oliver), is the most commonly used traditional Chinese medicine (TCM) in Min County, China, and has been demonstrated to exert blood-supplementing effects for thousands of years (Hua et al. 2017; Tian et al. 2017; Zhou et al. 2018). In China, AS slices are often simmered in soup for nourishing and tonifying blood. And some studies found the blood-supplementing effect of WASD was significantly enhanced (Zhao et al. 2006; Wang et al. 2012; Zhan et al. 2013; Ji et al. 2018; Peng et al. 2018).

Decoction is the main form of drug application in the clinical practice of Chinese medicine (Gong et al. 2015). It is reported that AS decoction has two effective components, ferulic acid (Xie and Liu 2013; Shinjyo et al. 2018) and polysaccharide (Liu et al. 2010; Hou et al. 2012; Wang et al. 2018), both related to blood tonic effect and are also found in other medicinal plants. In addition, caffeic acid is also an effective component of AS. It has been reported that caffeic acid also has a blood-supplementing effect (Lun et al. 2008; Song et al. 2009).

However, a systematic screening study of the blood-supplementing effect of each extraction fraction in AS decoction is lacking (Dong et al. 2005). To promote the development and application of WAS in the pharmaceutical industry, we optimized the preparation technology of WASD through response surface methodology (RSM), using ferulic acid and polysaccharide contents as evaluation indexes and liquid–solid ratio, soaking time, and extraction time as independent variables. According to the polar sizes of the main chemical components in WASD, ethyl acetate, *n*-butanol, and water fractions were obtained from...
WASD through ultrasonic-assisted extraction. Then, the blood-supplementing effects of the different extraction fractions (ethyl acetate, n-butanol, and water) of WASD were compared, and the best blood-supplementing active fraction of WASD was selected. The main active components of the blood-supplementing fractions were analysed.

Materials and methods

Instruments and materials

The following instruments were used: Agilent 1260 high-performance liquid chromatography system (Agilent, USA), DM type inner heat glass fibre electrothermal sleeve (Shandong Zhen Cheng Yong Xing instrument factory), YP1102H type analysis balance (Shanghai Precision Science Co., Ltd.), Lambda 7 SHIMADZU UV/visible spectrophotometer (Japan), RE-6000 rotary evaporator (Shanghai Yarong Biochemical Instrument Factory), and KQ-360DE type numerical control ultrasonic cleaner.

Glucose (20160824, Sinopharm Chemical Reagent Co., Ltd), ferulic acid (8J38-74DQ, National Institutes for Food and Drug control), caffic acid (110885-201603, National Institutes for Food and Drug control), and yellow rice wine (QS330615040013, Shaoxing Baita Brew Beer Co., Ltd) were used.

Preparation of WASD

AS roots were purchased from Min County, Gansu Province, China in January 2016, and authenticated by Dr. Yanming Wei (College of Veterinary Medicine, Gansu Agricultural University, Lanzhou, China). A voucher specimen was stored in the herbarium centre of Gansu Agricultural University (AS root, WAS201601001). The roots were decontaminated, cleaned, humidified, sliced, and dried. Sliced AS roots were mixed thoroughly in yellow rice wine, braised, fried until yellowish colour, and cooled according to the Pharmacopoeia Commission of People’s Republic of China and Gansu Processing Standard of TCM (Hua et al. 2014). Every 1 kg of Angelica sinensis is equal to 0.1 kg of yellow rice wine (200 mL/L).

Experimental animals

A total of 80 Kunming mice (body weight, 23 ± 2 g) were purchased from Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences. All the animal welfare and experimental procedures follow the guidelines for animal care and use and approved by the Animal Ethics Committee of Gansu Agricultural University.

Determination of the content of crude polysaccharide in WASD

Through the phenol sulphuric acid method (Chen et al. 2015), a glucose standard (100 mg) was accurately weighed, and 100 mL of water was added as the mother liquid. Then, glucose standard solutions with concentrations of 20, 40, 60, 80, 100, and 120 μg/mL were prepared. A phenol solution (5%, 1.8 mL) and concentrated sulphuric acid (7.5 mL) were added slowly into the standard solutions of different concentrations, and absorbance (483 nm) was determined. The control sample was treated with distilled water for the same treatment. The absorbance value (A) of each concentration was measured at 483 nm, and the standard curve of glucose concentration was obtained.

Crude polysaccharide in WASD was obtained through water extraction and alcohol precipitation method (Wang et al. 2012). The dried powder of WASD (1 g) was accurately weighed and transferred to a triangle bottle, then 70% ethanol solution (30 mL) was added. The bottle was then sealed and left to stand for 12 h. The floating material was washed with absolute ethanol, acetone, and diethyl ether, then the crude polysaccharide from WAS (WASP) was obtained through vacuum drying. WASP (10 mg) was added to a 50 mL volumetric flask. Distilled water was added at a fixed volume. WASP was completely dissolved through ultrasonic treatment (40 kHz, 50 W, 30 min). The absorbance value (A) of the sample was measured through the phenol–sulphuric acid.

Determination of the content of ferulic acid in WASD

Chromatographic analysis was performed through HPLC on an Agilent ZORBAX SB-C18 column (4.6 mm × 150 mm, 5 μm) at 35 °C. The mobile phases comprised (A) acetonitrile and (B) 0.085% aqueous phosphoric acid in water with a ratio of 17:83. The sample was injected (20 μL, injection volume) onto the column and eluted at a flow rate of 0.8 mL/min. Ultraviolet detection was set to 316 nm (Shi et al. 2014).

Ferulic acid standard (5.7 mg) was accurately weighed and transferred to a 50 mL brown volumetric bottle. Methanol solution (70%) was added at a fixed volume as a mother liquid. Then, the standard solutions of ferulic acid with concentrations of 0.005, 0.007, 0.009, 0.011, and 0.023 mg/mL were prepared. After filtration with a 0.22-μm organic phase needle filter, the content was determined through HPLC. Thereafter, the standard curve was drawn and the regression equation was established.

Approximately 0.1 g of dried WASD powder and 70% methanol solution was added to a 10 mL volumetric flask. After the mixture was shaken, ultrasonic processing was performed for 30 min. Then, the mixture was diluted to a certain volume and dissolved completely, filtered into the sample bottle through a 0.22-μm organic phase needle filter. The content was determined by HPLC.

Determination of overall desirability (OD) value

‘Overall desirability’ (OD) is a value that reflects the overall effect. In this experiment, ferulic acid and polysaccharide content were normalized through the Hassan method to reflect the comprehensive effect of each index (Liang et al. 2013). The calculation formula 1 is as follows (Fu and Zhan 2017):

\[
D_l = \left( \frac{Y_l - Y_{\text{min}}}{Y_{\text{max}} - Y_{\text{min}}} \right) \quad (1)
\]

where \(Y_l\) is the actual measured value of the index, \(Y_{\text{max}}\) represents the maximum value of the index in a single experiment, and \(Y_{\text{min}}\) is the minimum value (Cai et al. 2018). After each index of \(D_l\) was calculated, the OD value was calculated according to the following formula 2 (Fu and Zhan 2017):

\[
OD = \left( d_1 \times d_2 \ldots d_n \right)^{1/n} \quad (2)
\]

Single-factor experiment

The three main independent variables that affected the extraction process of WASD were liquid–solid ratio, extraction time, and soaking time. In the experiment, the liquid–solid ratios were 4:1, 6:1, 8:1, 10:1, and 12:1, the soaking times were 0, 30, 60, 90, 120,
and 150 min, and the extraction times were 60, 90, 120, 150, and 180 min. A single factor experiment was carried out. The obtained decoction was concentrated and dried under vacuum to a constant weight, and the yield of the extract was calculated. The polysaccharide and ferulic acid contents were determined through the above methods. The optimal level of a single factor was selected according to the OD value (Luan et al. 2013; Yu et al. 2013).

**Optimum extraction conditions of WASD through RSM**

According to the results of the single-factor experiment, the factors and levels of the RSM design were determined. The trial version of Design-Expert 8.0.6 was used in selecting the Box–Behnken centre experiment and optimizing the extraction condition of WASD (Lee 2012; Li et al. 2018).

**Preparation of WASD and different extraction fractions according to optimum screening conditions**

Based on previous research, we selected the optimum preparation conditions to obtain WASD. With comparing and analysing the yields, ethyl acetate, n-butanol, and water fractions were extracted from WASD through ultrasonic-assisted extraction and saved.

**Screening of the best blood-supplementing active fraction of WASD**

**Animal grouping and modelling.** The 80 Kunming mice were randomly and equally divided into eight groups: normal control (NC), model (M), intervention groups with low and high doses of ethyl acetate fraction (EAL and EAH), intervention groups with low and high dose of n-butanol fraction (NBL and NBH), and intervention groups with low and high dose of water fraction (WL and WH) after adaptive feeding for three days. Each mouse in the intervening groups with high doses of the fractions received 10 g/kg extraction fractions of WASD by gavage, whereas each mouse in the intervention groups with low doses received 5 g/kg extraction fractions. NC and M received the same amount of distilled water. Intragastric administration was performed at 9 a.m. for 9 days. M and each intervention group received acetylphenyl hydrazine normal saline solution (20 and 40 mg/kg, respectively) subcutaneous injection at the 2nd day and 5th day. They also received cyclophosphamide normal saline solution (40 mg/kg) through intraperitoneal injection at the 9th day. Meanwhile, NC was injected with normal saline. The animal experiment programs for the establishment of the blood deficiency model and the treatment experiment were in accordance with the study before (He et al. 2018). The general observation and body mass of mice in different groups were analysed. At the end of the experiment, the mice in each group were weighed and then anaesthetized, and blood samples were extracted for blood routine examination requiring red blood cells (RBC), white blood cells (WBC), and haemoglobin (Hb) indexes. Finally, the spleens and thymi were extracted, and spleen and thymi indexes were calculated. The thymi were preserved in 10% formaldehyde solution.

**Thymi histological analysis using haematoxylin and eosin (H&E) staining.** Thymi tissues were embedded in paraffin block and sliced manually into 3.0 μm sections with a microtome. The tissues were stained using the haematoxylin and eosin (H&E) method, and the structures were observed and photographed with a digital microscope.

**Statistical analysis.** Statistical analysis was carried with SPSS16.0. Independent sample t-test was used for two groups and one-way ANOVA for multiple groups, and a p value of <0.05 indicated significant difference.

**Components analysis of the blood-supplementing fractions**

**Determination of polysaccharide content.** The crude polysaccharide content in WASD was determined according to the methods in the section ‘Determination of the content of crude polysaccharide in WASD’. Then, the content of reducing sugar in WASD was determined through the DNS method. Fructose solutions (1 mg/mL) with volumes of 100, 150, 200, 250, 300, 350, and 400 μL were precisely transferred to test tubes. The DNS chromogenic agent of 400 μL was added and fixed to a volume of 5 mL. The test tubes were heated in a boiling water bath for 5 min, then rapidly cooled to room temperature with flowing water. Absorbance was determined at 510 nm after 0.5 h at room temperature. The sample absorbency A was determined through the DNS method after three parallel tests. Polysaccharide content is equal to the content of crude polysaccharide minus the content of reducing sugar.

**Determination of caffeic acid and ferulic acid content.** The chromatographic analysis conditions were same as those in the section ‘Determination of the content of ferulic acid in WASD’. The accurate caffeic acid standard (10 mg) was dissolved with 70% methanol (chromatographically pure) until a 0.1 mg/mL solution was obtained. Caffeic acid standard solutions (40, 80, 120, 160, and 200 μL) were transferred with a pipette into volumetric bottles, and 70% methanol solution was added to each bottle at a fixed volume. A standard curve was made using peak areas and standard concentrations (μg/mL).

n-Butanol fraction (0.1944 g) and water fraction (0.2004 g) were added to a 10-mL volumetric flask, and 70% methanol (chromatographically pure) was added. The mixture was shaken, subjected to ultrasonic processing for 30–60 min, then filtered into a sample bottle through a 0.22-μm organic phase needle filter. The content of caffeic acid and ferulic acid was determined through HPLC.

**Results**

**Study on the optimum extraction process of WASD**

**Standard curve of glucose**

The regression equation was as follows: \( Y = 0.008X + 0.127 \) (\( R^2 = 0.9987 \)).

The absorbance value was linearly related to glucose concentrations ranging from 20 to 140 μg/mL.

**Standard curve of ferulic acid and the chromatogram of ferulic acid and sample**

The regression equation was as follows: \( Y = 115741.730X - 47.258 \) (\( R^2 = 0.9982 \)).

A good linear relationship between the peak area and ferulic acid concentration ranging from 0.005 to 0.023 mg/mL was observed.

As shown in Figure 1(A, B), ferulic acid content can be obtained using the regression equation according to the peak area in the chromatogram.
**Analysis results of single-factor experiment**

As shown in Figure 2, the maximum OD value was obtained at a liquid–solid ratio of 8:1, soaking time of 120 min, and extraction time of 150 min. Thus, these values were selected as the optimal values of the three factors.

**Analysis results of RSM**

**Factor level selection of RSM.** Based on a single factor experiment and the design principle of Box-Behnken’s central combination experiment, the design level of RSM is shown in Table 1.

The data in Table 2 were analysed through a regression approach, and a parametric regression model was established. The quadratic polynomial regression equation of response value OD on the three factors was obtained.

\[
Y = -6.31150 - 0.85165A + 0.023395B + 0.12596C + (7.9500 \times 10^{-4}AB) + 0.012066AC + (7.57083 \times 10^{-5}BC) - 0.063280A^2 - (1.68478 \times 10^{-4}B^2) - (7.94637 \times 10^{-4}C^2)
\]

The results of the variance analysis of the model and significance test of coefficients are listed in Table 3. The \(p\) value for the regression model was 0.0488 (<0.05), which indicated that the model was meaningful. The \(p\) value of the lack-of-fit test of 0.0595 (>0.05; Table 3) implied non-significant difference and the goodness of fit degree of the model. The residual may be caused by random error. Thus, the model and the above quadratic polynomial regression equation can be used in analysing and predicting the OD value.

The \(R^2\) value for correction coefficient of the model was 0.9284, indicating that 92.84% of the response value can be explained through the above quadratic polynomial regression equation. The coefficient significance test results of the regression model showed that the interaction between factors not only had a certain impact on the OD value but also had no simple linear relations.

Table 1. Factors and levels in the RSM design.

| Factors (symbol, units) | Levels |  
|-------------------------|--------|
| Liquid–solid ratio (A, mL/g) | 7:1 | 8:1 | 9:1 |
| Soaking time (B, min) | 90 | 120 | 150 |
| Extraction time (C, min) | 130 | 150 | 170 |

Figure 1. (A, B) Chromatogram of ferulic acid standard and sample (A: ferulic acid standard; B: sample).

Figure 2. The results of single factor experiments.
The following figures (Figures 3(A,B), 4(a, b), and 5(a, b)) showed the other two factors and its interaction impacted on the OD value when one factor was fixed in the liquid–solid ratio, soaking and extraction time.

As shown in Figure 3(A,B), the OD value increased initially and then decreased with increasing liquid–solid ratio and soaking time at a fixed extraction time (150 min). Within a range of 114–126 min, the OD value increased initially and then decreased with increasing soaking time at a constant liquid–solid ratio. As the OD value increased initially and then decreased with increasing liquid–solid ratio increasing at a constant soaking time, a maximum value was obtained at 146–154 min of soaking time. The elliptical contour map shows the region at which the interaction between liquid–solid ratio and soaking time exhibited little effect on the OD value. The regression model of 0.0265 showed significant difference (p < 0.05), and it was in accordance with the response surface map.

In Figure 5(a, b), the OD value increased first and then decreased with increasing soaking and extraction times at a liquid–solid ratio of 8:1 mL/g. At a constant soaking time, the maximum OD value was obtained at 146–154 min of extraction time. At a constant extraction time, the maximum OD value was obtained at 146–154 min of soaking time. The elliptical contour indicated that the interaction between soaking and extraction times had no significant effect on the OD value, consistent with the regression model results.

### Analysis of response surface and contour

The following figures (Figures 3(A,B), 4(a, b), and 5(a, b)) showed the extraction time, and the maximum OD value was obtained between 7.5:1 and 8.5:1 mL/g. The results showed that the contour line was a gradient line, indicating that the interaction between liquid–solid ratio and extraction time exhibited little effect on the OD value. The regression model of 0.0265 showed significant difference (p < 0.05), and it was in accordance with the response surface map.

In Figure 5(a, b), the OD value increased first and then decreased with increasing soaking and extraction times at a liquid–solid ratio of 8:1 mL/g. At a constant soaking time, the maximum OD value was obtained at 146–154 min of extraction time. At a constant extraction time, the maximum OD value was obtained at 146–154 min of soaking time. The elliptical contour indicated that the interaction between soaking and extraction times had no significant effect on the OD value, consistent with the regression model results.

### Determination of the best extraction conditions and the verification test

After the partial derivative was obtained, \( A = 7.69, B = 119.78, \) and \( C = 143.35. \) Thus, the optimum extraction conditions were as follows: liquid–solid ratio of 7.69:1 mL/g, soaking time of 119.78 min, and extraction time of 143.35 min. The optimal OD value was 0.8437.

An extraction experiment was performed under the above optimal extraction conditions. The results showed that the actual experimental OD value was similar with the theoretical OD value, thus verifying the feasibility and rationality of the above
model and indicating that the RSM has good reliability, repeatability, and practicability.

Screening of the best blood-supplementing fraction in WASD

General observation. Compared with the mice in the NC group, the mice in the M group had no lustre of fur, no eye reaction, and no spirit; lacked blood in the ears and tails; and tended to stay together. Compared with the mice in the M group, the mice in the intervention groups showed good mentality and quick reactions, and their tails and ears had normal colours and the lustre fur and quick reaction. The mice in the WH group were the most closely to the mice in the NC group, followed by the NBH group.

Body mass observation. The results showed that the body mass change trend of mice in the WH group were the closest to that in the NC group, followed by the NBH group (Figure 6).

Main organ index analyses. In Table 4, compared with the NC group, the spleen and thymi indexes of mice in the M group significantly decreased ($p < 0.05$). Compared with the M group, the spleen and thymi indexes in the high-dose intervention groups significantly increased ($p < 0.05$). The spleen and thymi indexes in the WH and NBH groups significantly increased compared with those in the EAH group ($p < 0.05$). The spleen and thymi indexes increased significantly in the NBH group compared with those in the NBL group ($p < 0.05$).

Main blood routine index analysis. In Table 5, the results showed that RBC, WBC, and Hb in the M group significantly decreased compared with those in the NC group ($p < 0.05$). RBC, WBC, and Hb in each intervention group increased significantly compared with those in the M group ($p < 0.05$). RBC and Hb in the WH and NBH groups significantly increased compared with those in the EAH group ($p < 0.05$). RBC, WBC, and Hb in the
Histological observation of thymi. The mouse thymus in the NC group had no significant histologic lesions (Figure 7(A)). The cortex and medulla were clearly demarcated and complete and had clear structures. However, the thymus of the mice in the M group severely atrophied, the lymphocytes in the cortices were greatly reduced, fat degeneration was severe, and the boundaries between the cortices and medullas were blurred (Figure 7(B)). The WH group (Figure 7(C)) was the closest to the NC group, and the lowest number of pathological changes was observed in the thymi tissues, followed by that in the NBH group (Figure 7(D)) and the WL group (Figure 7(E)), indicating that the water and n-butanol fractions of WASD had better effects. The pathological changes in the thymi in the NBL group (Figure 7(F)), EAH (Figure 7(G)), and EAL (Figure 7(H)) groups were severe, indicating that the EAH fraction and low dosage of n-butanol fraction of WASD exhibited a poor intervention effect.

Components analysis of the blood-supplementing fractions. No polysaccharide was detected in the n-butanol fraction of WASD. The total sugar content of crude polysaccharide of the water fraction (1 mg) was 628.94 μg/mg, and the reducing sugar content was 376.35 μg/mg, and thus the polysaccharide content was 252.56 μg/mg. The HPLC analysis results showed that ferulic acid was not found in the water fraction. In the n-butanol fraction, the content of ferulic acid was 0.806 μg/mg. The regression equation of the standard curve of caffeic acid was as follows:

\[ Y = 12.5756077X - 8.1417265 \quad (R^2 = 0.99575) \]

(Y: peak area; X: concentration)

The HPLC analysis results showed that the contents of caffeic acid of the n-butanol and water fractions were 1.187 and 0.346 μg/mg, respectively.

Discussion

Study and analysis on the optimum extraction process of WASD

Water decoction is the main form of TCM used, and its cost is low. In the different processed products of *Angelica sinensis*, WAS is an important and popular kind of blood tonic medicine (Gong 2017; Jia 2017) and has been used in AS blood-supplementing oral liquid (Zhao et al. 2006; Gao et al. 2011). RSM has enormous advantages in the extraction study of active ingredients from TCM (Xue et al. 2017; Qian et al. 2019). Therefore, the optimal extraction process of WASD (liquid-solid ratio of 7.69:1 mL/g, soaking time of 119.78 min, and extraction time of 143.35 min) was obtained through RSM, and the contents of ferulic acid and crude polysaccharide were used. Our study is an innovative research on the development and utilisation of *Angelica sinensis*, and it can lay a theoretical foundation for the development and application of WAS in the pharmaceutical industry.

Screening and components analysis of the better blood-supplementing fractions of WASD

Replication of blood deficiency model

Blood deficiency is a common syndrome in traditional Chinese (Veterinary) medicine. Various methods for replicating this model are available, and each method has its advantages and disadvantages (Gao et al. 2003; Zeng et al. 2018). For example, blood loss method can directly reduce blood cells in the body, but cannot accurately determine the amount of blood loss (Li et al. 2012; Gautam et al. 2013). The chemical damage method, including the haemolytic and hemopoiesis-restraining types and their combination, improves blood deficiency models, but the maintenance time of blood deficiency state is long (Liu et al. 2012; Zhu et al. 2011). The radiation method is fast and effective, but the degree of blood deficiency cannot be controlled (Zhang et al. 2018). Therefore, the method of replicating blood deficiency model is mainly the model made with combined chemistry injury.

The mice in the M group had the same anaemic symptoms as the mice in the NC group, and RBC, WBC, and HB contents significantly decreased (p < 0.05), indicating that the blood deficiency model was successfully replicated.

### Table 4. Effects of various fractions of WASD on main organs indexes in blood deficiency mice.

| Group | Spleen index (%) | Thymus index (%) |
|-------|-----------------|-----------------|
| NC    | 3.00 ± 0.13     | 0.0035 ± 0.0003 |
| M     | 1.07 ± 0.7      | 9.61 ± 0.65     |
| EAL   | 2.38 ± 0.12     | 0.0026 ± 0.0009 |
| EAH   | 2.47 ± 1.45     | 136.67 ± 5.73   |
| NBL   | 2.03 ± 0.53     | 133 ± 13.46     |
| NBH   | 2.7 ± 0.57      | 151.75 ± 4.97   |
| WH    | 2.07 ± 0.78     | 134.67 ± 1.7    |
| WL    | 0.0050 ± 0.0017 | 0.0034 ± 0.0007 |

Note. NC represents the normal control group; M represents the model group; EAL and EAH represent the intervention groups with low and high doses of ethyl acetate fraction respectively; NBL and NBH represent the intervention groups with low and high doses of n-butanol fraction respectively; WL and WH represent the intervention groups with low and high doses of water fraction respectively; *represents the comparison with the NC; †represents the comparison with M; ‡represents the comparison with EAH; ††represents the comparison with WL; ¥represents the comparison with NBL.

### Table 5. The trend of blood routine changes in each group of mice (mean ± SD).

| Group | WBC (10^9/L) | RBC (10^12/L) | Hb (g/L) |
|-------|--------------|--------------|----------|
| NC    | 3.38 ± 1.23  | 9.98 ± 0.75  | 146.33 ± 10.77 |
| M     | 1.07 ± 0.7   | 9.61 ± 0.65  | 136.67 ± 5.73  |
| EAL   | 2.38 ± 0.12  | 8.11 ± 0.81  | 136.2 ± 14.88  |
| EAH   | 2.47 ± 1.45  | 8.40 ± 0.45  | 135.67 ± 5.73  |
| NBL   | 2.03 ± 0.53  | 7.99 ± 0.65  | 133 ± 13.46    |
| NBH   | 2.7 ± 0.57   | 9.61 ± 0.65  | 151.75 ± 4.97  |
| WH    | 2.07 ± 0.78  | 8.53 ± 0.23  | 134.67 ± 1.7   |
| WL    | 0.0050 ± 0.0017 | 0.0034 ± 0.0007 |

Note. NC represents the normal control group; M represents the model group; EAL and EAH represent the intervention groups with low and high doses of ethyl acetate fraction respectively; NBL and NBH represent the intervention groups with low and high doses of n-butanol fraction respectively; WL and WH represent the intervention groups with low and high doses of water fraction respectively; *represents the comparison with the NC; †represents the comparison with M; ‡represents the comparison with EAH; ††represents the comparison with WL; ¥represents the comparison with NBL.
Intervention effect of the different fraction of WASD

The results of general observation, blood routine, body mass, main organ indexes, and histological observation of thymis showed that the mice in each intervention group had different degrees of recovery compared with the mice in the M group. The mice in the WH group showed the closest performance to the NC group, followed by the NBH group. This result indicated that the intervention of the water and n-butanol fractions had better effect on blood-deficient mice and had a dose-effect relationship. These results are expected to be confirmed by in vitro experiment in the further studies.

Components analysis of the better blood-supplementing fractions of WASD

Through HPLC analysis and other methods, the main blood-supplementing active components in the best blood-supplementing fractions of WASD were analysed. The water fraction had the highest polysaccharide content (252.565 μg/mg), and the caffeic acid was 0.346 μg/mg. Ferulic acid was not detected. In the n-butanol fraction, ferulic acid was 0.806 μg/mg, caffeic acid was 1.187 μg/mg, and polysaccharide was not detected. Obviously, the blood-supplementing effect of the water and n-butanol fractions of WASD was positively correlated with the contents of polysaccharide, ferulic acid, and caffeic acid, consistent with the previous reports (Zheng and Wang 2001; Zhang et al. 2007; Yang et al. 2008; Lee et al. 2012; Li et al. 2015; Ji et al. 2018). The detailed blood-supplementing effect mechanisms of the two fractions are in progress.

Conclusions

The single factor experiment, the Hassan method and RSM were used to optimize the extraction process of WASD, and an OD value of 0.8437 was obtained under the optimal extraction conditions (liquid-solid ratio of 7.69:1 mL/g, soaking time of 119.78 min, and extraction time of 143.35 min). The water fraction (5 or 10 g/kg) and n-butanol fraction (10 g/kg) were the best blood-supplementing fractions in WASD. In the water fraction, the content of polysaccharide was 252.565 μg/mg, caffeic acid was 0.346 μg/mg, and ferulic acid was not detected. In the n-butanol fraction, the ferulic acid content was 0.806 μg/mg, caffeic acid was 1.187 μg/mg, and polysaccharide was not detected. This study lays a theoretical foundation for the subsequent development and application of Angelica sinensis processed with yellow rice wine in the pharmaceutical industry and offers a model for pharmaceutical development. If Angelica sinensis extracts are made into a buccal tablet or other dosage form for anaemia patients, post-operative patients, and postpartum women, then the decoction with a tedious process, can be replaced by buccal tablets or other dosage forms.
Disclosure statement

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