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Optimization of Purification Technology and Antioxidant Activity of Total Flavonoids from Sea Buckthorn Leaves

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Abstract: China is rich in sea buckthorn (*Hippophae rhamnoides L.*) resources and a large amount of sea buckthorn fruit is used to extract oil and juice every year. However, Sea Buckthorn Leaves (SBL), which are full of flavonoids, are discarded as waste. To make full use of resources and turn waste into valuables, the optimal purification process of flavonoids from SBL was studied and the antioxidant activities of Crude SBL Flavonoids (CFSBL) and Purified Flavonoids (PFSBL) were compared. The macroporous resin which was most suitable for the purification of CFSBL was selected by comparing the flavonoid content, adsorption rate, desorption rate, and recovery rate in eluant. The purification process of CFSBL was optimized by a single factor, Analytic Hierarchy Process (AHP) and Response Surface Methodology (RSM). 1,1-Diphenyl-2-Picrylhydrazyl (DPPH), hydroxyl radical, and reducing power methods were used to determine the antioxidant activity of CFSBL and PFSBL, respectively. AB-8 macroporous resin was optimized for purification and the optimal process was determined by RSM combined with AHP: The ethanol concentration was 82.93%, and the sample concentration and elution velocity were 0.09 and 1.71 mL/min, respectively. The purity of PFSBL was 84.2%, which was 6.53 times higher than that before purification and the recovery rate was 69.5%. The scavenging IC_{50} of DPPH, hydroxyl radical, and reducing the power of CFSBL were 0.016, 1.501, and 0.146 mg/mL, respectively. PFSBL were 0.002, 1.131 and 0.051 mg/mL, respectively. The antioxidant activity of PFSBL was improved compared with that of CFSBL. SBL can be considered a potential source of antioxidants in food and industry. The purification process in this study can enhance the antioxidant activity of SBL.

Keywords: Sea Buckthorn Leaves, Flavonoids, Analytic Hierarchy Process, Response Surface, Antioxidant Activity

Introduction

Sea buckthorn (*Hippophae rhamnoides L. subsp. Sinensis*) is a deciduous shrub of the genus *Hippophae* in *Elaeagnaceae*, also known as vinegar willow, *Elaeagnaceae* date, acid thorn, etc. (Ilhan *et al*., 2021). Native to Eurasian Mainland, especially in China (S Périno-Issartier *et al*., 2011), accounting for it accounts for more than 90% of the global sea buckthorn resources. Sea buckthorn is an essential edible and medicinal plant (Suryakumar and Gupta, 2011), which has traditionally been used to protect soil and water. Hundreds of bioactive substances were found in sea buckthorn, including abundant flavonoids and terpenoids (Zeb, 2004).

Compared with berries and seeds, Sea Buckthorn Leaves (SBL) are an inexpensive and unexploited byproduct (Michel *et al*., 2012). The chemical components of SBL include flavonoids, terpenoids, steroids, volatile oils, organic acids, phenols, and sugars, among which flavonoids are one of the main active components (Cho *et al*., 2014). SBL has attracted considerable attention due to its functional activity (Maisuthisakul *et al*., 2007). As the safety of synthetic antioxidants has been questioned, the search for safe and efficient natural antioxidants has become an important research direction for food additives (Pundir *et al*., 2021).

The crude extract of natural products has complex components and often contains some impurities, which have no activity or may sometimes cause some side effects. There are many traditional purification methods and it is necessary to explore the method suitable for industrial production (Xi *et al*., 2015). So the research on the purification of active ingredients has become crucial and has become a hot spot in application development. There are many ways to purify flavonoids. Macroporous adsorption resin has the advantages of large adsorption capacity, fast adsorption speed, simple regeneration, simple recovery, long service life, and so on (Wan *et al*., 2014).

TL Saaty, an American scientist, put forward the analytic hierarchy process in the 1970 s (Saaty, 2001). It is a way of combining problem-solving decisions with a specific approach to the solution and it uses the experience of decision makers to judge each project. The importance of the criteria can be achieved, the weight given to each decision plan is appropriate and the value and loss of each block can be judged by using a scale (Saaty, 2008). Response Surface Analysis (RSM) is a statistical method to obtain the optimal process parameters by analyzing the regression equation and fitting the functional relationship between factors and response values with multiple quadratic regression equations. It is widely used in the field of production and scientific research (Cheng *et al*., 2020).

In recent years, there have been many studies on the extraction of flavonoids from sea buckthorn, but there are few reports on the purification process of flavonoids from SBL and the specific analysis of the contents of various components and antioxidant properties. SBL is rich in flavonoids, but they are abandoned as waste, which not only wastes resources but also pollutes the environment. It is very significant to study flavonoids in SBL (Ma *et al*., 2016).

Therefore, the purpose of this study was to determine the appropriate range of ethanol concentration, sample concentration, elution velocity, and elution volume variables by single factor test and to evaluate the purification conditions by combining AHP and RSM. The recovery rate, adsorption rate, and desorption rate were weighted (w_1, w_2, w_3) and the comprehensive score of the best process was carried out. The changes in antioxidant activity of CFSBL and PFSBL were determined by DPPH, hydroxyl radical, and reducing ability.

Materials and Methods

Materials

Sea buckthorn was collected in September 2021 in Longtan District, Jilin City, Jilin Province. The voucher specimen (20210925) was preserved in the herbarium of Jilin Engineering Research Center for Agricultural Resources and Comprehensive Utilization, Jilin Institute of Chemical Technology, Jilin, China. The purity of chemicals and reagents was the analytical grade. Macroporous resin NKA-Ⅱ、X-5、D101、AB-8 and polyamide were purchased from Tianjin Yunkai Resin Technology Co., Ltd. (Tianjin, China). 1,1-Diphenyl-2-Picrylhydrazyl (DPPH), Hydrogen Peroxide (OH), and potassium ferricyanide (reduction force) (all are Aladdin, Holy City, Shanghai, China) and L-ascorbic acid (V_C, Shanghai Holy Land).

Methods

Extraction of Total Flavonoids

Pick fresh young leaves of sea buckthorn, spread them out, and dry them to dry for 24 h to make them lose water dehydrate. The SBL of a certain quality were taken and 80% ethanol was added at the rate of 1:20 and extracted for 1 h each time, twice in total. The filtrates were merged and concentrated by rotary evaporator, the CFSBL was obtained and then the sample is freeze-dried, and ground into powder for standby.

Screening of Five Macroporous Adsorption Resins

Pretreatment: five different macroporous adsorption resins (D101, AB-8, X-5, NKA-Ⅱ, polyamide) were immersed in 95% ethanol overnight to make them swell before use and washed with 95% ethanol to no white turbidities and then washed with distilled water to no alcohol flavor, soaked in distilled water, standby application to make the distilled water liquid level higher than the resin liquid level (Jiang *et al*., 2020).

Screening of macroporous adsorption resins: Weigh five resins for each 3 g, added 40 mL of total flavonoids solution of SBL, and adsorbed on a shaking table for 6 h statically. Then 40 mL of 80% ethanol was added and determine the content of total flavonoids in the filtrate by shaking for 6 h. 7 g of resin was taken, 20 mL of sample solution was added and the resin was dynamically adsorbed for 2 h. It was found that 4 times column volume used 80% ethanol elution and determine the content of total flavonoids in the eluent. The adsorption rate, desorption rate, and recovery rate of the eluents were calculated and compared by formulas (Liu *et al*., 2020). The adsorption rate of CFSBL was calculated as follows:

$$Ae = \left(C_0 - C_e\right) / C_0 \times 100\% \quad (1)$$

Desorption rate of CFSBL:

$$De = C_d V_d \ / \left(C_0 - C_e\right) V_0 \times 100\% \quad (2)$$

In the type:

C_0: The sample Concentration (mg/mL)
C_e: Adsorption Concentration (mg/mL)
C_d: Adsorption Concentration (mg/mL)
V_d: Elution Volume (mL)
V_0: Sample Volume (mL)

$$Recovery\ rate = Ae \times De \quad (3)$$

Purification of Total Flavonoids From SBL Single Factor Experiment

The SBL ethanol extract was concentrated at 15 mL. The effects of ethanol concentration (50, 60, 70, 80, 90%), elution rate (1,2,3,4,5 mL/min), sample concentration (0.1, 0.2, 0.3, 0.4, 0.5 mg/mL) and elution volume on the adsorption rate, desorption rate and recovery of SBL were investigated. When one factor was changed, the other three factors were fixed.

Analytic Hierarchy Process (AHP) for Weight Calculation

The priority weights of recovery rate w_1, adsorption rate w_2, and desorption rate w_3 were calculated by using the scaling method of judgment matrix element a_{ij}. This is shown in Table 1.

Firstly, define the problem, determine the analysis objectives and establish a "pyramid" hierarchy.

The calculation method of consistency index C. R. is as follows:

$$CI = (\lambda - n)/(n-1) \quad (4)$$

When, *CI*=0, there is complete consistency; When *CI* approaches 0, there is satisfactory consistency; The larger the *CI*, the greater the inconsistency.

The random consistency index RI is introduced to measure the size of CI:

$$RI = \left(CI_1 + CI_2 + ...CI_n\right)/n \quad (5)$$

According to the formula, the random consistency index *RI* is related to the order of the judgment matrix. The larger the order of the matrix, the greater the possibility of random deviation of the consistency. The corresponding relationship is shown in Table 2.

Since random bias may also lead to consistency deviation, it is necessary to compare CI with random consistency index RI when checking whether the judgment matrix has satisfactory consistency. The formula is as follows:

$$CR = CI / RI \quad (6)$$

In general, when, *CR*<0.1, the matrix is considered to pass the consistency test; otherwise, the consistency is not satisfactory. Initial weight coefficient:

$$W_1' = \sqrt[n]{a_{i1}\, a_{i2}\, a_{i3} \cdots a_{in}} \quad (7)$$

In the type: a: value in the matrix; m: the number of goals. Normalized weight coefficient:

$$W_i = W_i' / \sum_{i=1}^{n} W_i' \quad (8)$$

Experimental Design of Response Surface Optimization

Response Surface Analysis (RSM) was combined with a single factor experiment to select reasonable ethanol concentration, elution velocity, sample loading concentration, and elution volume. According to the combined experimental design principle of the Box-Behnken center and the results of a single factor experiment, three factors ethanol, concentration (A), sample concentration (B), and elution velocity (C), were selected. The adsorption rate, desorption rate, and recovery rate of CFSBL were integrated into a comprehensive score through the AHP as the final target response value. The statistical analysis software Design-Expert V 8.0.6.1 Trial was used to conduct the Box-Behnken central combination test Design and 17 response surface analysis experiments with three factors and three levels were established. Seventeen points were factorial experiments and five points were central experiments. Quadratic multinomial regression equation fitting and optimization analysis are carried out (Luo and Yang, 2004). Experimental factors and coding level design are shown in Table 3.

In Vitro Antioxidant Capacity

To verify the feasibility of the optimal purification process, DPPH, hydroxyl radical and reducing ability, and free radical scavenging were carried out with positive control (V_C) of CFSBL and PFSBL.

The method of DPPH radical scavenging activity was determined by referring to the method of Wang *et al*. (2017) with some modifications(Wang *et al*., 2017). 2.0 mL of different concentrations of flavonoids before and after purification were mixed with 2 mL of DPPH (0.1 mmol/L) anhydrous ethanol solution and the absorbance value A_i was determined at 517 nm after 35 min of dark reaction at room temperature. The Model control group was mixed with an equal volume of DPPH and anhydrous ethanol solution and the absorbance value A_0 was determined. In the blank control group, an equal volume of the test solution was mixed with an anhydrous ethanol solution to determine the absorbance value A_j. Ascorbic acid (V_C) was used to replace the sample solution to test the antioxidant activity of the positive control. Blank zero was set with anhydrous ethanol. The DPPH radical scavenging rate was calculated as follows:

$$Scavenging\ rate\% = 1 - (A_i - A_j)/A_0 \times 100\% \quad (9)$$

The method of hydroxyl radical scavenging activity was determined by referring to the method of (Hongyan *et al*., 2018). The powder samples of total flavonoids from SBL were weighed accurately to prepare a test solution of different concentrations. 1 mL of the test solution, 1.0 mL of phenanthrene 0.75 mmol/L, 2 mL of PBS (pH = 7.4) and 1 mL of distilled water were mixed and then added 1.0 mL 0.75 mmoL $FeSO_4$ and 1.0 mL 0.012% H_2O_2 were mixed. With water-bath temperature at 37℃ for 60 min. A_p was measured at 511 nm; Distilled water was used instead of H_2O_2 to determine the absorbance value A_b; As was determined by using the test solution instead of distilled water; V_C was used as the positive control. The hydroxyl radical scavenging rate was calculated as follows:

$$Scavenging\ rate\% = \left(A_s - A_p\right) / \left(A_b - A_p\right) \times 100\% \quad (10)$$

The method of reducing ability was determined by referring to the method with some modifications (Adibi *et al.*, 2011). 1 mL of flavonoids samples were added into 2.5 mL PBS (pH = 6.6) and 2.5 mL 1% potassium ferricyanide solution. Put it in a water bath at 50°C for 20 min, add 2.5 mL of 10% trichloroacetic acid and shake well. Centrifugation at 3000 r/min for 10 min. Take 2.5 mL supernatant, add 0.5 mL 0.1% ferric chloride solution and 2.5 mL distilled water, and shake well. The absorbance of the reaction solution measured at 700 nm was denoted as A_i and that of the control group was denoted as A_0 with distilled water instead of the sample. The reducing ability scavenging rate was calculated as follows:

$$Scavenging\ rate\ \% = 1 - A_i / A_0 \times 100\ \% \quad (11)$$

The flow chart of the experimental methods is shown in Fig. 1 and 2.

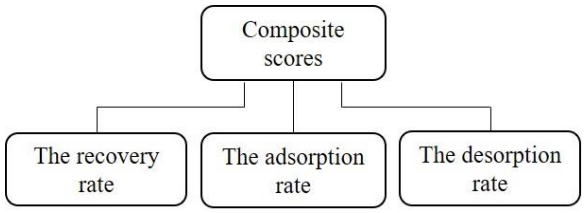

Fig. 1: Pyramid hierarchy

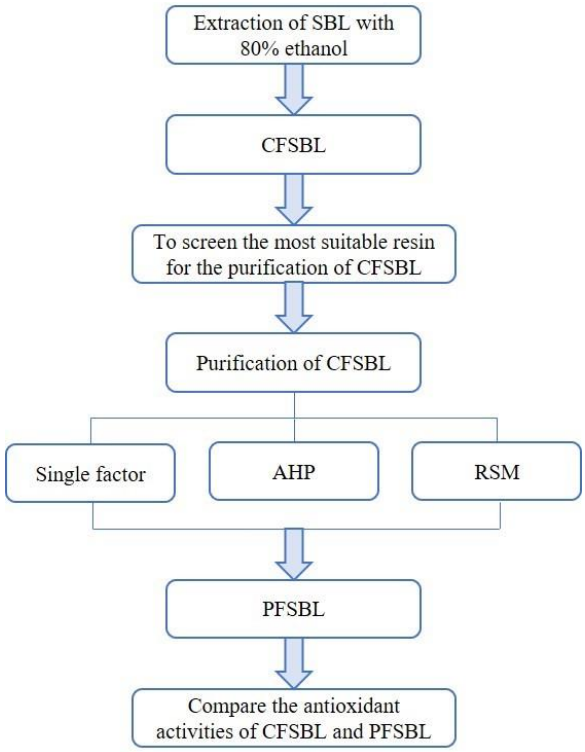

Fig. 2: The flow chart of the experiment methods

Results and Discussion

Selection of the Best Resins

Macroporous adsorption resin has a special structure and it has remarkable physical and chemical stability because of its unique porous structure (Liu *et al.*, 2010). And one-way ANOVA of AB-8 was compared with four other macroporous adsorption resins. The results are shown in Fig. 3, the adsorption rates of CFSBL on AB-8 and D101 resins were significantly higher than those on other resins and the desorption rate on AB-8 and polyamide resins was better.

Table 1: Proportional scale table

The factor I over factor j	Quantitative values
As important	1
A little important	3
More important	5
Highly important	7
Extremely important	9
The middle value of two adjacent judgments	2, 4, 6, 8

Table 2: Average random consistency index RI standard value

Order number	1	2	3	4	6	7	8	9	10
RI	0	0	0.85	0.90	1.24	1.32	1.41	1.45	1.49

Table 3: Experimental factors and coding level design

Factors	code	level		
Ethanol concentration (%)	X_1	A	70.00	80.0	90.0
Sample concentration (mg/ml)	X_2	B	0.05	0.1	0.2
Elution velocity (ml/min)	X_3	C	1.00	2.0	3.0

But the recovery rate of AB-8 was higher. In summary, the comparison between resins was significant in the unidirectional analysis.

The AB-8 resin has a large specific surface area and pore diameter, which is suitable for the adsorption of various hydrophobic components. It has favorable properties in terms of adsorption capacity, ease of elution, and adsorption kinetics. Stable to heat, organic solvents, and general conditions of use of acid, alkali, so long service life (Zhou *et al.*, 2010). It can barely adsorb protein, sugar, inorganic acid, alkali, salt, and small hydrophilic organic matter, so it can separate the general components from these substances. AB-8 has the best performance in terms of adsorption rate, desorption rate, and recovery rate. Therefore, AB-8 was selected as the best resin to purify total flavonoids from SBL.

Single Factor Primary Screening

As shown in Fig. 4 (a), the effect of ethanol concentration on the recovery rate of CFSBL. The higher the concentration of ethanol, the higher the recovery. When the concentration of ethanol reached 80%, the recovery rate reached the maximum. However, with the continuous increase of the concentration of ethanol, the recovery rate tended to decrease. This is because the increase in the concentration of ethanol increases the solubility of CFSBL, which is conducive to the leaching of flavonoid compounds.

As shown in Fig. 4 (b), the effect of elution velocity on the recovery rate of CFSBL. With the increase of elution velocity, the recovery of CFSBL increased firstly and then decreased. When the elution velocity was 2 mL/min, the recovery rate reached the maximum. This is because when the elution velocity increases to a certain extent, the flavonoids and ethanol on the resin will be fully dissolved, making the recovery rate reach the maximum. However, with the increase in elution velocity, the flavonoids on the resin may not be fully dissolved by ethanol, resulting in incomplete elution and the recovery rate of CFSBL was decreased.

As shown in Fig. 4 (c), the effect of sample concentration on the recovery rate of CFSBL. With the increase in sample concentration, the recovery of CFSBL firstly increased and then decreased and the recovery reached the maximum when the sample concentration was 0.1 mg/mL. Because of the excessive sample concentration, the resin adsorption is supersaturated, resulting in the loss of most of the flavonoids, resulting in a low recovery rate.

As shown in Fig. 4 (d), the effect of elution volume on the recovery rate of CFSBL. The recovery rate increased with the increase of elution volume. The reason may be that with the increase of eluent, some impurities adsorbe on the resin will be eluted off, leading to the increase of CFSBL, but the purity will decrease accordingly, which will affect the purification effect. Therefore, according to the experimental experience, when the elution volume was 4 BV, almost all the flavonoids on the resin would be washed off, so the elution volume was 4 BV.

AHP Calculating the Weights

(1) Calculate the matrix and get the maximum eigenvector
$\lambda max = 3.1632$,
$CI = (3.1632-3)/(3-1) = 0.0816$,
$RI = 0.0816/0.85 = 0.096 < 0.1$,
that is, the matrix passes the consistency test
(2) Initial weight coefficient calculation results:
$W_1' = 1.817$, $W_2' = 0.405$, $W_3' = 1.357$
(3) Normalized weight coefficient:
$\{\text{Recovery rate, adsorption rate, desorption rate}\}^T = \{0.508, 0.113, 0.379\}^T$

The results of the single-factor experiment can be intuitively seen through the results of the analytic level analysis. The concentration of ethanol was 80%, the elution velocity was 2 mL/min and the loading concentration was 0.1 mg/mL. Response surface experiments were carried out using the single factor results.

Experimental Design of Response Surface Optimization

Model Fitting and Statistical Analysis

Statistical analysis was performed using a desk expert software v8.0.6.21. After multiple regression fitting, the second-order polynomial equation was used to optimize the purification process conditions related to independent parameters and comprehensive evaluation components of CFSBL. Ethanol concentration (A), sample concentration (B), and elution velocity (C) were taken as independent variables and the comprehensive score of CFSBL was taken as the response value. The quadratic regression model equation of the predicted value of the comprehensive score of flavonoids from SBL and the coded value of A, B, and C were obtained:

$$Y = +68.47+3.93\times A-2.73\times B-5.03\times C-5.26\times A\times B-2.37\times A\times C-0.74\times B\times C-11.50\times A^2-4.98\times B^2-9.31\times C^2.$$

Experimental results of response surface analysis are shown in Table 4-6. Further analysis of variance showed that there was an interaction between each factor and the response target value. From the overall model of this paper, we can see A^2, B^2, and C^2 are conspicuous, A is conspicuous in the first term factor and AB has conspicuous influence in the interaction term, while other items are not. By comparing the *P* values, it was found that the order of the single factor influencing the model

was the elution velocity> ethanol concentration > sample concentration. According to the determination coefficient R^2=0.9401, the reliability of the model is good. According to the missing term P = 0.0654>0.05 indicates that the fitting loss of the model is not conspicuous. The results well demonstrated that there was a strong correlation between the experimental values and the theoretical values deduced by fitting with the corresponding polynomial. The results showed that the model prediction is reliable and repeatable in the range of parameters (Cheng *et al*., 2020). The results of variance analysis showed that the precision of the model is 9.211>4. It means that the model formula can be predicted after any combination of ethanol concentration, elution velocity, and sample concentration. According to the experimental data, this model was appropriate for the analysis and prediction of the purification process of CFSBL.

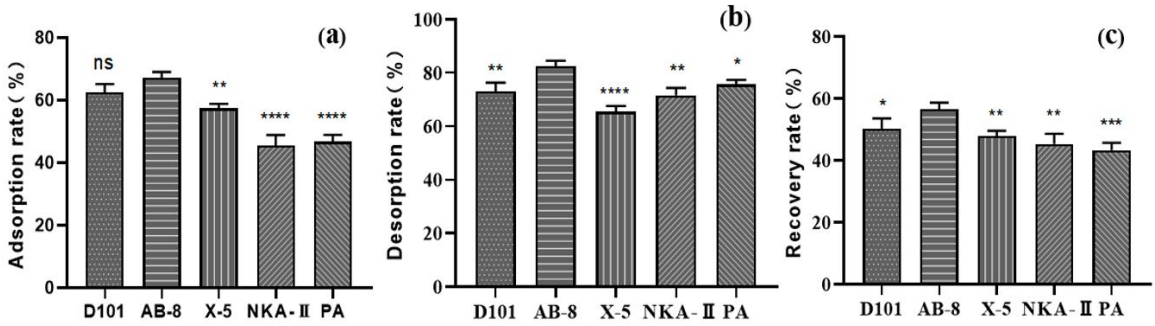

Fig. 3: Investigation results of five different resins (adsorption rate (a), desorption rate (b), and recovery rate (c))

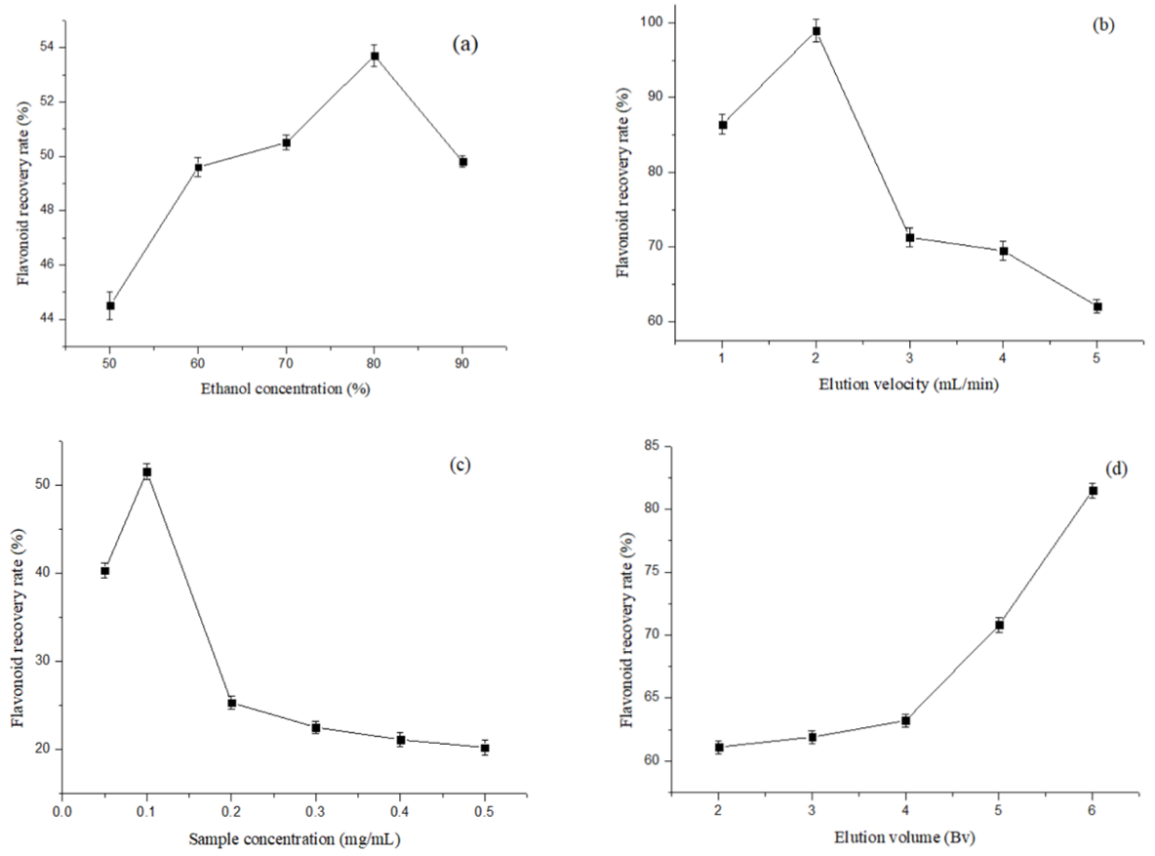

Fig. 4: Effects of four factors on recovery rate. (a) Ethanol concentration; (b) Elution velocity; (d) Sample concentration; (d) Elution volume

Response Surface Analysis

Response surface graph analysis is used to form a three-dimensional space diagram for specific response target values and corresponding independent variables, which could reflect the influence of various factors on response target values (Akalın *et al*., 2015). The 3D response surface map can be analyzed to find out the corresponding interaction factors of each factor in the reaction process, to obtain the corresponding response surface map. The corresponding response surface parameters can be used to judge the influence efficiency.

As shown in Fig. 5 (A), the surface diagram of the interaction between ethanol concentration and sample concentration must remain unchanged after the elution velocity was fixed at 1.71 mL/min. It can be seen from the RSM in Fig. 5 that when the concentration of ethanol is between 70 and 80%, the surface presents an obvious slope and when it reaches the highest point, the surface appears to be flat, indicating that the concentration of ethanol has a great impact on the purification process and there is a significant difference. It can be seen from Fig. 5 (B) that the sample concentration must be maintained at 0.09 mg/mL and the concentration of ethanol at 80% and the two factors must be fixed unchanged. It can be found that in the response surface figure, when the elution velocity is 1 mL/min, the surface has an evident slope, indicating that the elution velocity has a significant impact on the purification effect. It can be seen from Fig. 5 (C) that when the sample concentration is between 0.05 - 0.1 mg/mL, the surface rises slowly, indicating that the interaction of this factor is insensitive to the purification effect of CFSBL.

Verification of Prediction Models

Through the further analysis of the fitting linear equation, the optimal purification parameters were finally obtained through software analysis. Under the optimal purification conditions, ethanol concentration was 82.93%, elution velocity was 1.71 mL/min and sample concentration was 0.09 mg/mL. In theory, the comprehensive score of CFSBL should be 70.33%.

To verify the accuracy of the model prediction, the optimal purification conditions were repeated five times to obtain the purity of 74.85, 72.97, 76.54, 73.58, and 77.95% of PFSBL, respectively. The purity of PFSBL was 75.17%±1.6% in five experiments. It can be seen that the final experimental results were the same as the predicted value provided by the software, which indicated that the model used in the experiment was applied to the purification conditions of CFSBL.

In vitro Antioxidant Capacity

The results are shown in Fig. 6 (A), DPPH clear rate of CFSBL and PFSBL showed a dose-effect relationship in the range of 0.001~0.008mg/mL and the scavenging rate of PFSBL was close to Vc. PFSBL (IC_{50} = 0.002 mg/mL), Vc (IC_{50} = 0.0045 mg/mL) and CFSBL (IC_{50} = 0.016mg/mL) were calculated by SPSS software. It can be seen from Fig. 6 (D) that PFSBL has significantly higher scavenging activity for DPPH free radicals than CFSBL, but there is no significant difference with Vc (P>0.05).

Hydroxyl radicals can be produced in the H_2O_2/Fe^{2+} system through the Fenton reaction (Kadri *et al*., 2013). The results are shown in Fig. 6 (B), the clear rate of hydroxyl radical of PFSBL was 32.07±1.90~81.63±2.11% in the range of 0.6-2.0 mg/ mL. PFSBL (IC_{50} = 1.131 mg/mL), Vc (IC_{50} = 0.817 mg/ mL) and CFSBL (IC_{50} = 1.50 mg/mL) were calculated by SPSS software. The scavenging activity of flavonoids from SBL was concentration-dependent and the scavenging activity of PFSBL was significantly higher than that of CFSBL (P<0.0001) and there were also significant differences between PFSBL and V_C (P<0.0001) (Fig. 6 D).

When the free radical scavenging activity is determined by the ferric ion reducing capacity method, potassium ferricyanide reacts with the sample and is reduced to potassium ferrocyanide and then react with Fe^{3+} to form Prussian blue (Zhao *et al*., 2008). The results are shown in Fig. 6 (C), the clear rate of PFSBL in the range of 0.02-0.1 mg/mL was 34.42±1.47~85.89±1.38%. It can be seen that there is a significant correlation between the reduction activity and the concentration of iron ions. PFSBL (IC_{50} = 0.051 mg/mL), Vc (IC_{50} = 0.060 mg/mL) and CFSBL (IC_{50} = 0.146 mg/mL) were calculated by SPSS software. The reduction ability of PFSBL was significantly higher than that of CFSBL in a concentration-dependent relationship (P<0.05) and the scavenging activity of PFSBL was not statistically different from that of VC (P>0.05) (Fig.6 D).

Rutin, quercetin-3-O-glucoside, quercetin, kaempferol, and isorhamnetin are five major flavonoid compounds in the SBL extracts, which possess stronger antioxidant activities since they have a relatively low oxidation potential 3-hydroxyl group, which can be oxidized irreversibly thus avoiding redox cycling (Guo *et al*., 2017; Cui *et al*., 2018). The purity of flavonoids in SBL increased from 12.9 to 84.24% and the antioxidant activity also increased significantly. In conclusion, flavonoids in SBL have good antioxidant activity. Therefore, SBL can be used as natural antioxidants in medicine, functional food, and cosmetics.

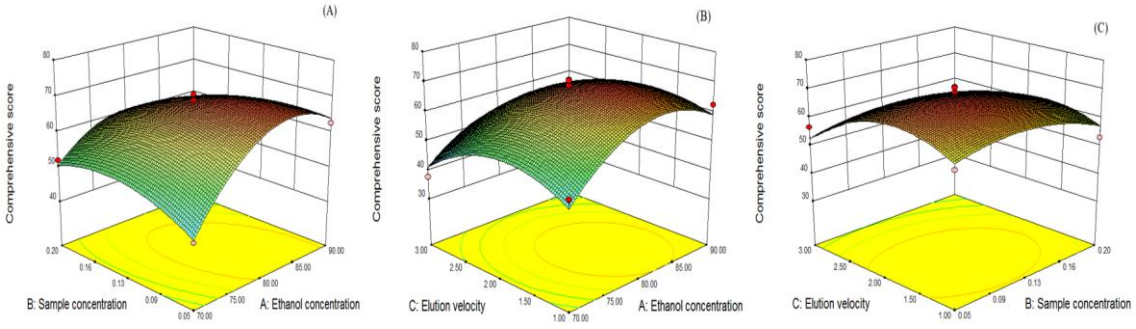

Fig. 5: Effects of three factors on the comprehensive score (ethanol concentration (A), sample concentration (B), and elution velocity (C))

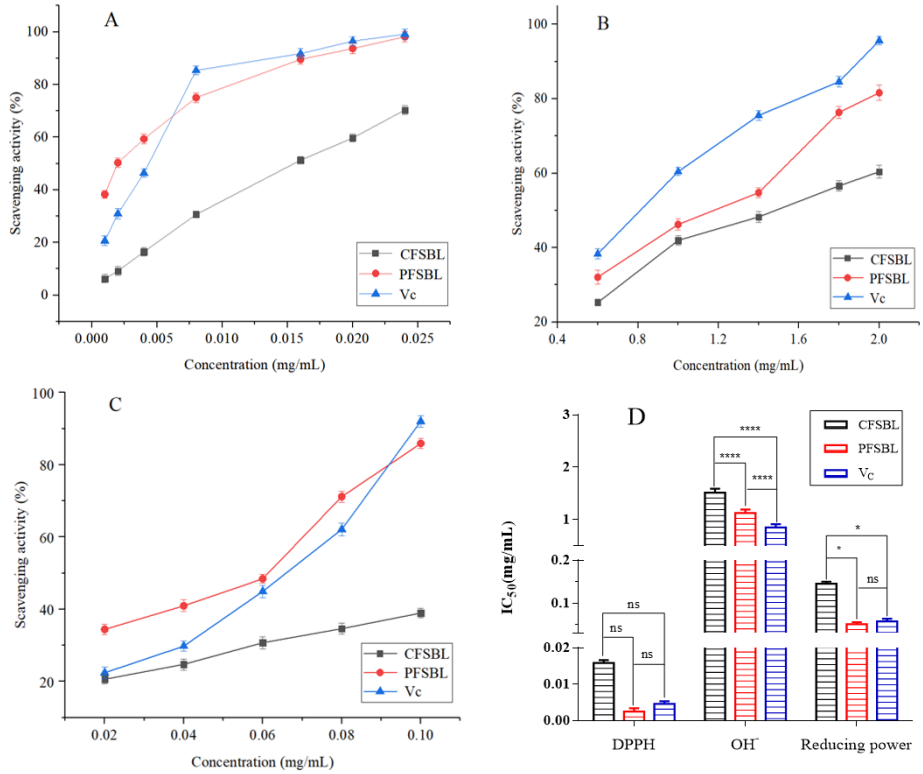

Fig. 6: Results of antioxidant activity of PFSBL and CFSBL (DPPH assay (A), Hydroxyl radical assay (B), Reducing power assay (C), IC50 value (D))

Table 4: Flavonoid purification scale table

	The recovery rates	The adsorption rates	The desorption rates
The recovery rate W_1	1	3	2
The adsorption rate W_2	1/3	1	1/5
The desorption rate W_3	1/2	5	1

Table 5: AHP results

Ethanol concentration (%)	Comprehensive score	Elution velocity (ml/min)	Comprehensive score	Sample concentration (mg/ml)	Comprehensive score
50	51.79892	1	89.48326	0.05	53.63155
60	57.40307	2	99.72834	0.10	60.76065
70	58.08343	3	82.30649	0.20	33.40447
80	61.03315	4	80.47097	0.30	30.97537
90	57.67947	5	71.98407	0.40	29.06071
-		-		0.50	26.60388

Table 6: Experimental results of response surface analysis

No.	A	B	C	The comprehensive score (%)
1	80.00	3.00	0.20	47.7010
2	70.00	3.00	0.13	37.7049
3	80.00	1.00	0.05	59.1816
4	80.00	2.00	0.13	70.6548
5	90.00	3.00	0.13	41.4373
6	80.00	2.00	0.13	68.9102
7	70.00	2.00	0.05	44.7687
8	80.00	2.00	0.13	66.2709
9	90.00	2.00	0.05	62.5495
10	70.00	2.00	0.20	51.9500
11	90.00	2.00	0.20	48.6845
12	80.00	1.00	0.20	53.0928
13	90.00	1.00	0.13	62.3658
14	70.00	1.00	0.13	49.1468
15	80.00	2.00	0.13	70.5379
16	80.00	2.00	0.13	65.9660
17	80.00	3.00	0.05	56.7359

Conclusion

In this study, AB-8 macroporous adsorption resin, AHP index evaluation system, and response surface design were used to determine the optimal purification conditions of CFSBL. The PFSBL showed a dose-dependent scavenging activity close to Vc, which was significantly higher than that of the CFSBL, indicating that the purified flavonoids in the solution contained abundant antioxidant components. It is promising and potential to be processed into natural medicines, health products, and cosmetics. In this way, not only will the technical issues of multi-index purification be effectively eliminated, but also sea buckthorn leaf flavonoids will be oriented to be applied to industrial production.

Nomenclature

SBL: Sea Buckthorn Leaves
CFSBL: The Crude Flavonoids of Sea Buckthorn Leaves
PFSBL:The Purified Flavonoids of Sea Buckthorn Leaves
AHP: Analytic Hierarchy Process
RSM: Response Surface Methodology
DPPH: 1,1-Diphenyl-2-Picrylhydrazyl
OH: Hydrogen peroxide

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Author's Contributions

Jiale Shan: Responsible for experimental data analysis, chart processing, and manuscript writing.

Yang Liu: Collection and summary of experimental data.

Pu Zhou: Responsible for experimental operation.

Hongli Zhou: Experimental design and modification of manuscript.

Peng Wan: Revision of manuscript.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and that no ethical issues are involved.

References

Adibi, H., Mojarrad, J. S., Asgharloo, H., & Zarrini, G. (2011). Synthesis, in vitro antimicrobial, and antioxidant activities of chalcone and flavone derivatives holding allylic substitutions. *Medicinal Chemistry Research*, *20*(8), 1318-1324. DOI.org/10.1007/s00044-010-9474-3

Akalın, M. K., Tekin, K., Akyüz, M., & Karagöz, S. (2015). Sage oil extraction and optimization by response surface methodology. *Industrial Crops and Products*, *76*, 829-835. DOI.org/10.1016/j.indcrop.2015.08.005

Cheng, X., Cheng, Y., Zhang, N., Zhao, S., Cui, H., & Zhou, H. (2020). Purification of flavonoids from Carex meyeriana Kunth based on AHP and RSM: Composition analysis, antioxidant and antimicrobial activity. *Industrial Crops and Products*, *157*, 112900. DOI.org/10.1016/j.indcrop.2020.112900

Cho, H., Cho, E., Jung, H., Yi, H. C., Lee, B., & Hwang, K. T. (2014). Antioxidant activities of sea buckthorn leaf tea extracts compared with green tea extracts. *Food Science and Biotechnology*, *23*(4), 1295-1303.DOI.ORG/10.1007/s10068-014-0178-1

Cui, Q., Liu, J. Z., Wang, L. T., Kang, Y. F., Meng, Y., Jiao, J., & Fu, Y. J. (2018). Sustainable deep eutectic solvents preparation and their efficiency in extraction and enrichment of main bioactive flavonoids from sea buckthorn leaves. *Journal of Cleaner Production*, *184*, 826-835. DOI.org/10.1016/j.jclepro.2018.02.295

Guo, R., Chang, X., Guo, X., Brennan, C. S., Li, T., Fu, X., & Liu, R. H. (2017). Phenolic compounds, antioxidant activity, antiproliferative activity, and bioaccessibility of Sea buckthorn (*Hippophae rhamnoides L*.) berries as affected by in vitro digestion. *Food & Function*, *8*(11), 4229-4240. DOI.org/10.1039/C7FO00917H

Hongyan, L. I., Ruihai, L. I., Lin, Z. H. A. O., & Yang, C. A. O. (2018). Inhibitory Effects of ginseng water extract on hydroxyl radical. *Medicinal Plant*, *9*(4). DOI.org/CNKI:SUN:MDPT.0.2018-04-033

Ilhan, G., Gundogdu, M., Karlović, K., Židovec, V., Vokurka, A., & Ercişli, S. (2021). Main agro-morphological and biochemical berry characteristics of wild-grown sea buckthorn (*Hippophae rhamnoides L.* ssp. caucasica Rousi) genotypes in Turkey. *Sustainability*, *13*(3), 1198. DOI.ORG/10.3390/SU13031198

Jiang, H., Li, J., Chen, L., & Wang, Z. (2020). Adsorption and desorption of chlorogenic acid by macroporous adsorbent resins during extraction of Eucommia ulmoides leaves. *Industrial Crops and Products*, *149*, 112336. DOI.org/10.1016/j.indcrop.2020.112336

Kadri, H., Djilani, S. E., & Djilani, A. (2013). Phytochemical constituents, antioxidant activity, total phenolic and flavonoid contents of Arisarum vulgare seeds. *Acta Scientiarum Polonorum Technologia Alimentaria*, *12*(2), 169-173. https://www.food.actapol.net/pub/4_2_2013.pdf

Liu, J., Meng, J., Du, J., Liu, X., Pu, Q., Di, D., & Chen, C. (2020). Preparative separation of flavonoids from Goji berries by mixed-mode macroporous adsorption resins and effect on Aβ-expressing and anti-aging genes. *Molecules*, *25*(15), 3511. DOI.org/10.3390/molecules25153511

Liu, Y., Liu, J., Chen, X., Liu, Y., & Di, D. (2010). Preparative separation and purification of lycopene from tomato skins extracts by macroporous adsorption resins. *Food Chemistry*, *123*(4), 1027-1034. DOI.org/10.1016/j.foodchem.2010.05.055

Luo, Z. Q., & Yang, S. L. (2004). Comparative study on several scales in AHP. *Systems Engineering-Theory & Practice*, *9*, 51-60. DOI.org/10.2116/analsci.20.717

Ma, X., Laaksonen, O., Zheng, J., Yang, W., Trépanier, M., Kallio, H., & Yang, B. (2016). Flavonol glycosides in berries of two major subspecies of sea buckthorn (Hippophaë *rhamnoides L*.) influence growth sites. *Food Chemistry*, *200*, 189-198. DOI.org/10.1016/j.foodchem.2016.01.036

Maisuthisakul, P., Suttajit, M., & Pongsawatmanit, R. (2007). Assessment of phenolic content and free radical-scavenging capacity of some Thai indigenous plants. *Food Chemistry*, *100*(4), 1409-1418. DOI.ORG/10.1016/j.foodchem.2005.11.032

Michel, T., Destandau, E., Le Floch, G., Lucchesi, M.E.,& Elfakir, C. (2012). Antimicrobial, antioxidant, and phytochemical investigations of sea buckthorn (Hippophaë *rhamnoides L*.) leaf, stem, root, and seed. *Food Chemistry*, *131*(3), 754-760. DOI.ORG/10.1016/J.FOODCHEM.2011.09.029

Périno-Issartier, S., Abert-Vian, M., & Chemat, F. (2011). Solvent-free microwave-assisted extraction of antioxidants from sea buckthorn (*Hippophae rhamnoides*) food by-products. *Food and Bioprocess Technology*, *4*(6), 1020-1028. DOI.ORG/10.1007/S11947-010-0438-X

Pundir, S., Garg, P., Dviwedi, A., Ali, A., Kapoor, V. K., Kapoor, D., ... & Negi, P. (2021). Ethnomedicinal uses, phytochemistry and dermatological effects of *Hippophae rhamnoides L*.: A review. *Journal of Ethnopharmacology*, *266*, 113434. DOI.ORG/10.1016/j.jep.2020.113434

Saaty, T. L. (2001). *Decision making for leaders: The analytic hierarchy process for decisions in a complex world*. RWS publications. DOI.ORG/10.1007/1-4020-0611-X_31

Saaty, T. L. (2008). Decision-making with the analytic hierarchy process. *International Journal of Services Sciences*, *1*(1), 83-98. DOI.org/10.1504/IJSSCI.2008.017590

Suryakumar, G., & Gupta, A. (2011). Medicinal and therapeutic potential of Sea buckthorn (*Hippophae rhamnoides L*.). *Journal of Ethnopharmacology*, *138*(2), 268-278. DOI.ORG/10.1016/J.JEP.2011.09.024

Wan, P., Sheng, Z., Han, Q., Zhao, Y., Cheng, G., & Li, Y. (2014). Enrichment and purification of total flavonoids from flos Populi extract with macroporous resins and evaluation of antioxidant activities in vitro. *Journal of Chromatography B*, *945*, 68-74. DOI.org/10.1016/j.jchromb.2013.11.033

Wang, R., Chang, Y., Tan, Z., & Li, F. (2017). A novel combined process for extracting, separating, and recovering flavonoids from flos *sophorae* immaturus. *Separation and Purification Technology*, *172*, 422-432. DOI.org/10.1016/j.seppur.2016.08.038

Xi, L., Mu, T., & Sun, H. (2015). Preparative purification of polyphenols from sweet potato (*Ipomoea* batatas L.) leaves by AB-8 macroporous resins. *Food Chemistry*, *172*, 166-174. DOI.org/10.1016/j.foodchem.2014.09.039

Zeb, A. (2004). Important therapeutic uses of sea buckthorn (*Hippophae*): A review. *Journal of Biological Sciences*, *4*(5), 687-693. DOI.ORG/10.3923/JBS.2004.687.693

Zhao, H., Fan, W., Dong, J., Lu, J., Chen, J., Shan, L.,...& Kong, W. (2008). Evaluation of antioxidant activities and total phenolic contents of typical malting barley varieties. *Food Chemistry*, *107*(1), 296-304. DOI.org/10.1016/j.foodchem.2007.08.018

Zhou, J. C., Feng, D. W., & Zheng, G. S. (2010). Extraction of sesamin from sesame oil using macroporous resin. *Journal of Food Engineering*, *100*(2), 289-293. DOI.org/10.1016/j.jfoodeng.2010.04.011