RESEARCH ARTICLE

Optimization of ultrasound and enzyme assisted hydrodistillation extraction of essential oil from *Schisandra chinensis* and its antioxidant activity

Zan Li, Shi Zhou, Yingxue Guo, Xiaoyi Chen, Yingli Liu, Zheji Zheng, Zhihao Hu, Siqi Li, Zijia Zhao, Min Yuan*

School of Pharmacy, Jiamusi University, Jiamusi, Heilongjiang, China.

Received: June 17, 2024; **accepted:** September 12, 2024.

Essential oils are complex mixtures of various secondary metabolites, mainly found in the fruits, seeds, and leaves of aromatic plants. Modern medical research demonstrates that it performs a wide range of biological activities such as anti-inflammatory, antibacterial, and antioxidant. Therefore, it is extensively used in the fields of medicine, cosmetics, and food industries. The essential oil is one of the important active components of *Schisandra chinensis* (Turcz.) Baill. and has various pharmacological properties. This study reported a novel method, ultrasound and enzyme assisted hydrodistillation (UEAH), for the extraction of essential oil. The single-factor experiments were used to investigate the effect of different parameters of pH, temperature, time, enzyme dosage on the extraction rate. A 3-level-3-factor Box-Behnken design of response surface was conducted to determine the optimal extraction conditions. The chemical components of essential oil were then quantitatively identified by gas chromatography-mass spectrometry (GC-MS). The antioxidant activity of essential oils obtained by UEAH and hydrodistillation was compared. The results showed that the optimal UEAH conditions were found to be an extraction time of 41.148 min, pH of 4.58, temperature of 56.248 ℃. The theoretical extraction rate under the optimized condition was 1.461%. The influence degree of the single factors on extraction rate was ranked as temperature > time > pH. The validation experiment once again proved the feasibility of this extraction technique. GC/MS analysis had identified a total of 40 compounds with the majority being terpenoids. Copaene (28.83%) was the major component followed by γ-terpinene (8.62%) and benzene,1-methyl-3-(1-methylethyl) (4.33%). The essential oil obtained by UEAH method exhibited excellent antioxidant activity, including prominent DPPH radical (EC_{50} = 0.939 ± 0.006 mg/mL), $ABTS^+$ radical (EC_{50} = 0.260 ± 0.007 mg/mL) scavenging effect and ferric reducing antioxidant power, which was higher than that of hydrodistillation. The findings indicated that UEAH method was simple and practical. More importantly, it could enhance the quality of essential oil.

Keywords: *Schisandra chinensis*; essential oil; ultrasound and enzyme assisted hydrodistillation; response surface methodology; GC/MS; antioxidant activity.

***Corresponding author:** Min Yuan, School of Pharmacy, Jiamusi University, Jiamusi 154007, Heilongjiang, China. Email: m15645326791@163.com.

Introduction

Schisandra chinensis (Turcz.) Baill., a perennial deciduous woody liana of the *Schisandraceae* family, is mainly located across China, Korea, and Japan. It is well known as a homologous plant of medicine and food that has excellent performance in healthcare and therapeutic effect [1, 2]. Many commercial healthcare products such as fruit wine, vinegar, and fruit tea have

already been developed to enhance physical function and improve the quality of life. Its fruit is also used to treat various diseases such as cough, cancer, parkinsonian syndrome, diabetes [3-6], which is due to the variety of active components contained in *Schisandra chinensis*, including polysaccharides, essential oils, lignin, and organic acids [7]. Multiple studies showed that lignin and polysaccharides were key substances and had received extensive attention [8-10]. *Schisandra chinensis* essential oil (SCEO), a volatile secondary metabolism, has been given the equal concern in recent years. The dominant components of SCEO are terpenoids and aromatic compounds [11, 12], which exhibit a range of pharmacological activities such as anti-inflammatory, antidepressant-like effect, and liver protection [13-15]. These remarkable traits make SCEO possess great potential for exploration in clinical practice. Therefore, it is urgent to develop a reasonable and efficient extraction method to improve resource utilization.

Several techniques had been documented for the extraction of essential oils including hydrodistillation extraction (HE) [16], supercritical fluid extraction (SFE) [17], organic solvent extraction (OSE) [18], microwave-assisted extraction (MAE) [19], and others. However, the above methods have their own drawbacks in practice, which results in certain restrictions in application. For example, the extraction efficiency of HE is relatively low and time-consuming despite its simple operation. SFE requires expensive instruments. OSE typically uses low-boiling materials like petroleum ether and carbon tetrachloride, which may cause environmental pollution. MAE is not suitable for the extraction of substances with poor thermal stability. Besides, enzyme-assisted extraction and ultrasound-assisted extraction are also applied in the extraction of essential oils. Enzyme-assisted extraction can effectively destroy the structure of cytoderm and facilitate the release of active components [20], which offers the benefits of energy conservation and environmental protection because of the mild conditions and absence of organic solvents. However, the efficiency of enzymolysis is not significantly better than that of conventional techniques. Ultrasound-assisted extraction fully utilizes the cavitation effect to destroy the cytoderm, thereby enhancing the efficiency of extraction process [21]. Ultrasound and enzyme assisted method involves the use of ultrasound and enzymes as pretreatment, thereby integrating the advantages of both methods. This method offers efficient and simple operation, ensuring the stability of the extracted materials. At present, the ultrasound and enzyme assisted method has been employed for the extraction of natural active substances. Wang *et al*. reported that this method was used to extract alkaloids from *Sophora alopecuroides* L [22], demonstrating its notable benefits in terms of high yield and resistance to degradation. Wei *et al*. applied ultrasound assisted aqueous enzymatic method to extract oil of *Cinnamomum camphora* seeds. The content of saturated fats obtained by this method was lower than that of Soxhlet extraction [23]. Vivek *et al*. investigated the synergistic effect of ultrasound and enzymolysis for the extraction of Sohiong (*Prunus nepalensis*) juice and found that the extraction time was reduced by 22 min compared to that of single enzymolysis [24].

According to previous investigations, the extraction technologies of SCEO included supercritical fluid [25], ionic liquid-based microwave-assisted extraction [26], solvent-free microwave extraction [27]. However, the extracted method of SCEO by ultrasound and enzyme assisted hydrodistillation (UEAH) has not been reported yet. This study reported a new extraction method for SCEO extraction. The extraction condition was optimized by response surface methodology. The chemical components were identified using gas chromatography-mass spectrometry (GC-MS). The antioxidant activity of SCEO extracted using the UEAH method was compared to that through hydrodistillation. The results of this study provided theoretical guidance for large-scale industrial production and the further development of SCEO.

Materials and methods

Extraction process and single factor experiments

30 g powder of *Schisandra chinensis* obtained from Langduoli Traditional Chinese Medicine Planting Co., Ltd (Hegang, Heilongjiang, China) was placed in a beaker containing distilled water for 1 h before adding 0.5% cellulase (Xia Sheng Enzyme Biotechnology Co., Ltd, Cangzhou, Hebei, China). Ultrasound and enzymolysis were carried out at 60℃ for 30 min, while the pH was set as the variables at 3.0, 3.5, 4.0, 4.5, and 5.0, respectively. The hydrodistillation method was conducted in accordance with the China Pharmacopoeia (China Medical Science and Technology Press, Beijing, China). The mixture of *Schisandra chinensis*, cellulase, and water was transferred to a single-neck round bottom flask. The top of the flask was connected to an essential oil extractor and then to a condenser. The mixture was kept in a boiling state by the DZTW heating mantle (Beijing Yongguangming Medical Instrument Co., Ltd, Beijing, China). Water vapor and essential oil ascended to the condenser where they were cooled into liquid that was subsequently collected in the essential oil extractor. The cock of the extractor was unscrewed, allowing the essential oil to flow out and be collected in a glass bottle. Anhydrous sodium sulfate (Guangzhou Haoying Chemical Technology Co., Ltd, Guangzhou, Guangdong, China) was added as a solid desiccant to the essential oil and stirred thoroughly before centrifugation. The dry essential oil was weighed to determine the quality. The experiment was repeated three times, and the extraction rate was calculated as follows.

Extraction rate (%) = (essential oil quality/powder quality) ×100%

SCEO was stored at 4℃. The effects of temperature at 45℃, 50℃, 55℃, 60℃, 65℃, enzyme dosage of 0.1%, 0.3%, 0.5%, 0.7%, 0.9%, and time of 20 min, 30 min, 40 min, 50 min, 60 min on the extraction rate were investigated. In the process of optimizing factors, it was guaranteed that only one factor was changed while the other factors remained unchanged.

Response surface experiment

Box-Behnken design was used to optimize selected factors. The experimental schemes of 3-level-3-factor were designed for analysis of variance, 3D response surface, and contour map using Design Expert 13 software (https://www.statease.com/software/design-expert/). The three factors involved were pH (A), temperature (B), and time (C), each of which had three different levels. For example, the three levels of pH were 4.0, 4.5, and 5.0, respectively. The three levels of temperature were 50℃, 55℃, and 60℃. The three levels of time were 30 min, 40 min, and 50 min. The extraction rate was taken as the dependent variable.

GC-MS analysis

GC-MS analysis was performed to identify the chemical components of SCEO using the HP 6890 gas chromatograph coupled with the HP 5973 mass selective detector (Agilent Technologies, Santa Clara, California, USA). The chromatograph was equipped with column of DB-5 quartz capillary (30 mm × 0.25 mm × film thickness 0.25 μm). The injection temperature was set at 250℃. The changes of programmed temperature were first maintained at 80℃ for 2 min, then increased to 120℃ at a rate of 5℃/min for 20 min, followed by an increase to 275℃ for 5 min. The temperatures of transmission line and gasification were 280℃ and 250℃, respectively. The flow rate of carrier gas (Helium) was 1 mL/min. For mass spectrometric conditions, the ionization voltage was set at 70 eV. The temperature of ion source was 250℃. The scanning range was obtained from 20 to 550 amu.

DPPH radical scavenging assay

The DPPH radical (2,2-diphenyl-1-picrylhydrazyl) scavenging assay was performed as described by Blois [28]. Briefly, 0.1 mL of 0.2 mmol/L DPPH methanolic solution (Shanghai Yuanye Biotechnology Co., Ltd, Shanghai, China) was mixed with the 0.1 mL sample methanolic solutions. The absorbance of the mixture was

measured using a microplate reader (Thermo Fisher Scientific, Waltham, MA, USA) at 518 nm after 30 min. The results were calculated as follows.

DPPH radical scavenging effect (%) = $(1 - A_1/A_0) \times 100\%$

where A_0 was the absorbance value of the DPPH and methanol. A_1 was the absorbance value of the DPPH and sample solutions.

$ABTS^+$ radical scavenging assay

The $ABTS^+$ radical (2'-azinobis-(3-ethylbenzthiazoline-6-sulphonate) scavenging assay was conducted as described by Kotora *et al*. with slight modifications [29]. Briefly, 0.1 mL sample solution was mixed with 0.1 mL $ABTS^+$ solution (0.05 mL of 7.4 mmol/L ABTS (Shanghai Yuanye Biotechnology Co., Ltd, Shanghai, China) and 0.05 mL of 2.6 mmol/L $K_2S_2O_8$ (Henan Mingzhixin Chemical Products Co., Ltd, Zhengzhou, Henan, China)). The mixture was then placed in the dark for 6 min. The absorbance of the mixture was measured at 734 nm. The results were calculated as follows.

$ABTS^+$ radical scavenging effect (%) = $(A_0 - A/A_0) \times 100\%$

where A_0 was the absorbance value of the $ABTS^+$ and methanol. A was the absorbance value of the $ABTS^+$ and sample solutions.

FRAP assay

The FRAP (ferric reducing antioxidant power) assay was measured using the method from Siddiq *et al*. [30]. Briefly, 1 mL sample solution was mixed with 2.5 mL of 0.2 mmol PBS buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide in 50℃ HH-S water bath (Zhengzhou Changcheng Science and Trade Co., Ltd, Zhengzhou, Henan, China) for 20 min. 2.5 mL of 10% trichloroacetic acid, 2.5 mL of distilled water, and 0.5 mL of 0.1% ferric chloride were then added. A 200 µL solution was taken from the mixture to measure the absorbance at 700 nm (A_1). The same process was carried out without samples (A_0). The results were calculated using the following formula.

Ferric reducing antioxidant power = $A_1 - A_0$

The results for antioxidant assays were expressed as EC_{50}. The EC_{50} represented the concentration at which the inhibition rate was 50%.

Statistical analysis

SPSS version 29 (IBM, Armonk, New York, USA) was used for data statistical analysis. All data were expressed as the mean ± standard deviation. The ANOVA test was performed for the statistical differences.

Results and discussion

Single factor experiments analysis

The effect of pH on the extraction rate of SCEO showed that the extraction rate gradually increased within the pH range of 3.0 to 4.5, indicating an enhanced role of cellulase in degrading cytoderms (Figure 1a). When the pH was 4.5, the highest amount of extraction was achieved. It was speculated that this might be attributed to the severe damage to the cytoderm, reducing the dissolution resistance of active substances. Subsequently, the extraction rate decreased with the increasing pH. Therefore, a pH of 4.5 was the most suitable for subsequent experiments. The influence of enzyme dosage on the extraction rate demonstrated that the extraction rate increased continuously with enzyme dosage ranging from 0.1% to 0.7% (Figure 1b), which could be explained by the fact that the increase in dosage improved the probability of contact between enzyme and substrate. When the dosage was 0.7%, the extraction rate reached the maximum, suggesting that the binding between the enzyme and the substrate had reached full saturation. When the enzyme dosage exceeded 0.7%, there was a slight decrease in extraction rate. The excessive aggregation of enzyme could block the through-hole of the cytoderm, leading to a

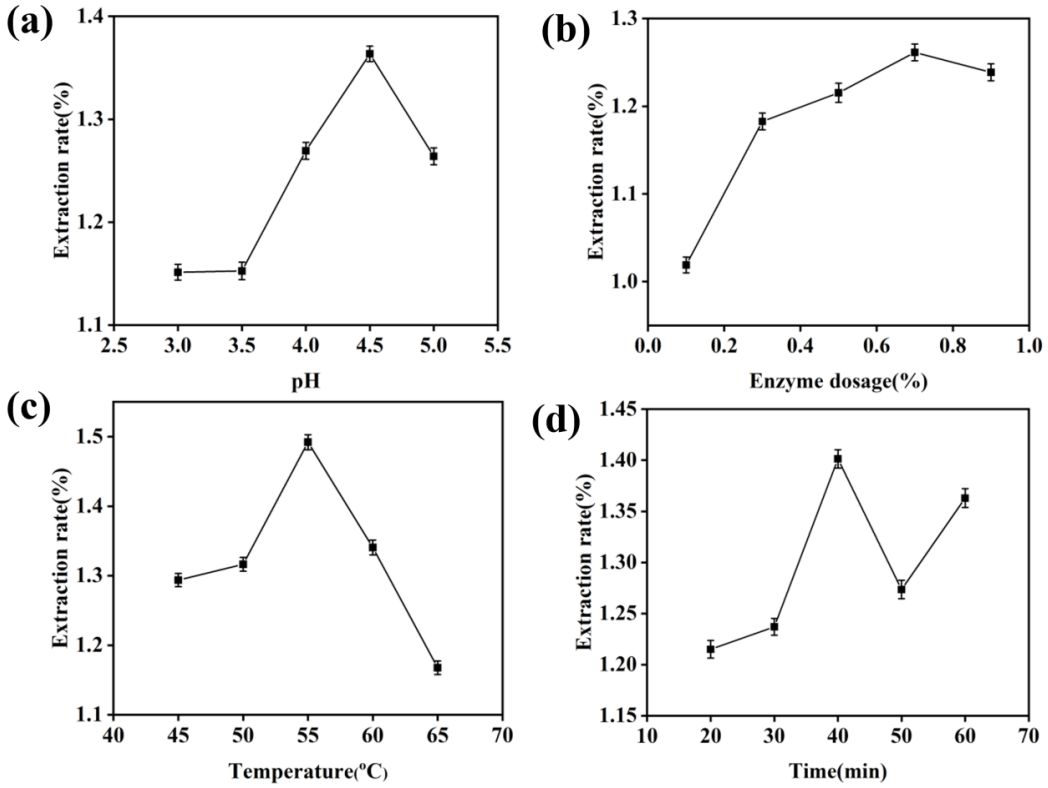

Figure 1. Effects of single factor on extraction rate of SCEO. (a): pH. (b): enzyme dosage. (c) temperature. (d) time.

decrease in the release of essential oil. Therefore, the enzyme dosage 0.7% might be considered suitable. The temperature had a certain impact on the extraction rate. A gradual increase in extraction rate was observed at temperature 45℃ to 55℃, suggesting that higher temperature might enhance the thermal motion of molecules and thereby improve the efficiency of enzymolysis (Figure 1c). The maximum extraction rate was achieved at 55℃. As the temperature continued to rise, the extraction rate began to decrease, which was attributed to the thermal-induced inactivation of enzyme and the degradation of components. Therefore, a temperature of 55℃ might be considered as optimal temperature. The time was also an important factor during SCEO extraction. During the time interval of 20 to 40 min, there was a corresponding increase in yield (Figure 1d). The highest extraction rate was obtained at 40 min due to the complete combination of enzyme and substrate, from which point extraction rate began to decline. When the time reached 50 min, the extraction rate demonstrated a further increase. The reason was that ultrasound facilitated the dissociation of the enzyme from its original substrate. Subsequently, the free enzyme was able to bind with a new substrate to initiate a new reaction. Overall, 40 min had been determined as the optimal time.

Model of the response surface and test of significance

According to the design principle of Box-Behnken center combination, a total of 17 experimental schemes were designed, and the different extraction rates were determined using those schemes. The quadratic polynomial equation was obtained through regression fitting as follows.

Y = -9.66138 + 1.92900A + 0.185025B + 0.072962C - 0.005100AB - 0.000950AC - 0.000320BC - 0.175000A2 - 0.001320B2 - 0.000615C2

where Y was extraction rate. A, B, C were pH, temperature, and time, respectively. The significance analysis showed the F value of 41.12 and the *P* value less than 0.0001, which provided strong evidence for the model's extreme significance, indicating a high level of agreement with the actual data. The *P* value of 0.5955 in lack of fit was greater than 0.05, suggesting that the mismatch term had no significant effect on the extraction rate. The results were less interfered by unknown factors, which could better reflect the relationship between the extraction rate and pH, temperature, time. Therefore, it was appropriate for this regression equation to predict and analyze actual results. The R^2 value of 0.9814 indicated that the model was capable of accurately explaining up to 98.14% of the variation. The results of R^2_{Adj} = 0.9576 (≥ 0.80) and CV% = 1.2% (< 5%) reflected the excellent stability and reliability of the test process. The significance of each factor (A, B, C), quadratic terms (A2, B2, C2), and interaction terms (AB, AC, BC) was also analyzed. The quadratic terms typically represented the squared terms of each factor. They were introduced to better fit the nonlinear relationship, allowing the model to more accurately reflect the real situation. The interaction terms denoted the effect of the combined single factors on the extraction rate. Each factor and quadratic terms were extremely significant at the level of $P < 0.01$. The interaction terms AB and BC exhibited significant effect as well ($P < 0.05$). However, interaction term AC showed insignificant effect ($P > 0.05$).

Interaction analysis of different factors

3D response surface and contour map could effectively demonstrate the interaction between the two factors. The steeper the surface, the greater the influence of this factor on extraction rate. In the contour map, an oval indicated a significant interaction, while a circle was not significant. The results showed that the temperature curve was steeper than that of pH and time, indicating that temperature had a greater influence on the extraction rate (Figures 2a and 2e). The influence of temperature was more pronounced than that of time (Figure 2e). It was evident that the effect of time was greater than pH (Figure 2c). Overall, the effect of single factor on extraction rate demonstrated that temperature > time > pH. The contour maps in Figures 2b and 2f were oval, indicating a significant interaction between the temperature and pH, as well as temperature and time. Figure 2d presented an approximate circle, implying that the interaction between pH and time was not significant. The above results were consistent with the variance analysis.

Validation experiment

The optimum conditions of SCEO were obtained using Design Expert 13 and pH of 4.58, temperature of 56.248℃, time of 41.148 min were identified as optimal conditions. Under optimal conditions, the theoretical yield was 1.461%. The parameters were then adjusted to pH of 4.6, temperature of 56℃, and time of 41 min when the experiments were conducted in laboratory practice for 5 times. The average extraction rate of obtained essential oil was 1.450%. The practical outcome was very close to the theoretical yield of the regression equation. Compared to the average yield of essential oil by hydrodistillation (1.02%), the yield of essential oil using UEAH method increased 0.43%. It was worthwhile to consider using UEAH technology for the extraction of SCEO.

Chemical component analysis

The SCEO was light yellow liquid with an aromatic odor. Its chemical components were identified by GC–MS, and the GC chromatogram was displayed in Figure 3. The 40 compounds eluted between 3 to 45 min accounted for 90.16% of the total components (Table 1). The content of copaene had the highest proportion of 28.83% followed by γ-terpinene of 8.62%, benzene,1-methyl-3-(1-methylethyl of 4.33%, (1R,2R,4S,6S,7S,8S)-8-lsopropyl-1-methyl-3-methylenethylenetricycli [4.4.0.02.7]decan-4-ol of 4.15%, and benzene,2-methoxy-1-methyl-4-(1-methylethyl) of 3.80%. The SCEO contained a higher amount of sesquiterpenoids and monoterpenes accounting for 43.64% and 18.74%, respectively. Terpenoids were known for their diverse pharmacological

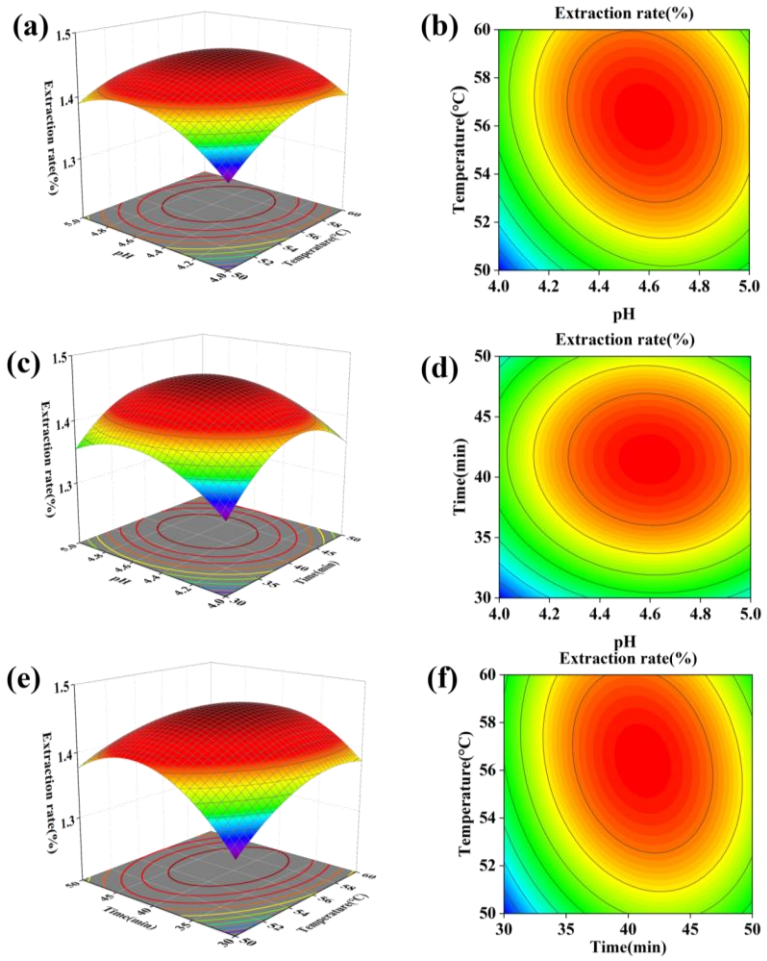

Figure 2. 3D response surface and contour maps of interaction between two factors. (a) and (b): effect of the interaction between pH and temperature. (c) and (d): effect of the interaction between pH and time. (e) and (f): effect of the interaction between temperature and time.

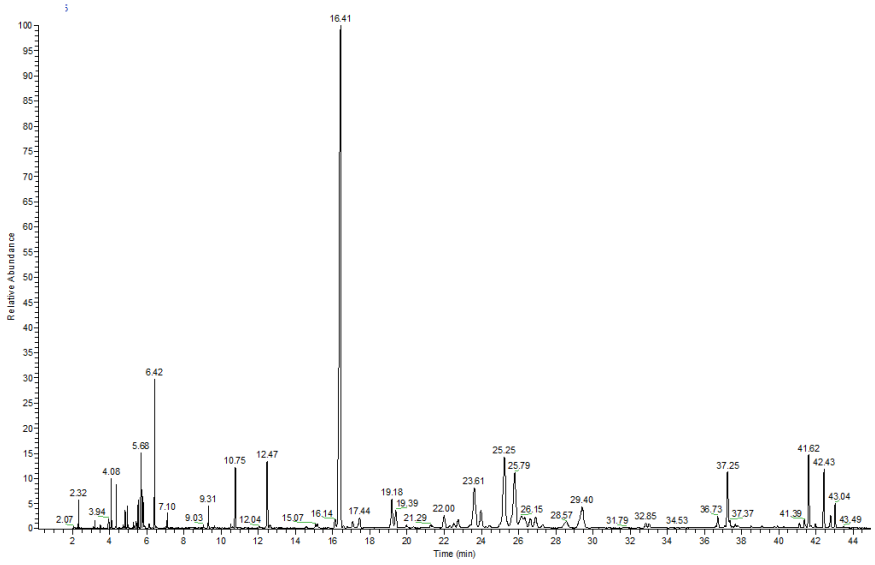

Figure 3. Total ion flow chart of GC-MS of SCEO.

Table 1. The chemical composition of SCEO.

Peak number	Compound	Chemical formula	Retention time (min)	Percentage (%)
1	p-xylene	C_8H_{10}	3.18	0.43
2	Bicyclo[3.1.0] hex-2-ene,2-methyl-5-(1-methylethyl)-	$C_{10}H_{16}$	3.93	0.53
3	Bicyclo [2.2.1] heptane, 2,2-dimethyl-3-methylene-, (1S)-	$C_{10}H_{16}$	4.34	2.49
4	Bicyclo [3.1.0] hexane, 4-methylene-1-(1-methylethyl)-	$C_{10}H_{16}$	4.81	1.03
5	Bicyclo [3.1.1] heptane, 6.6-dimethyl-2-methylene-, (1S)-	$C_{10}H_{16}$	4.94	1.26
6	3-carene	$C_{10}H_{16}$	5.41	0.36
7	(+)-4-carene	$C_{10}H_{16}$	5.52	1.64
8	Benzene,1-methyl-3-(1-methylethyl)	$C_{10}H_{14}$	5.69	4.33
9	D-liminene	$C_{10}H_{16}$	5.78	1.77
10	β-ocimene	$C_{10}H_{16}$	6.12	0.22
11	γ-terpinene	$C_{10}H_{16}$	6.41	8.62
12	2-carene	$C_{10}H_{16}$	7.08	0.82
13	endo-borneol	$C_8H_{18}O$	9.03	0.14
14	3-cyclohexen-1-ol,4-methyl-1-(1-methylethyl)-, (R)-	$C_8H_{18}O$	9.29	1.28
15	L-α-terpineol	$C_8H_{18}O$	9.62	0.12
16	2-octen-1-ol, 3, 7-dimethyl-	$C_{10}H_{20}O$	10.49	0.19
17	Benzene, 2-methoxy-1-methyl-4-(1-methylethyl)	$C_{11}H_{16}0$	10.74	3.52
18	Benzene, 2-methoxy-1-methyl-4-(1-methylethyl)	$C_{12}H_{20}0_2$	12.46	3.80
19	α-terpinyl acetate	$C_{12}H_{20}0_2$	15.07	0.18
20	1, 2, 4-metheno-lH-indene,octahydno-1, 7a-dimethyl-5-(1-methylethyl)-[1S-(1α, 2α, 3aβ, 4α, 5α, 7aβ, 8S*)]-	$C_{15}H_{24}$	16.13	0.38
21	Copaene	$C_{15}H_{24}$	16.38	28.83
22	(-)-β-bourbonene	$C_{15}H_{24}$	17.07	0.32
23	Bicyclo [5.30] decane, 2-methylene-5-(1-methylviny)-8-methyl	$C_{15}H_{24}$	17.45	0.53
24	(1R,3aS8aS)-7-isopropyl-1, 4-dimethtl-1, 2, 3, 3a, 6, 8a-hexahydro a zulene	$C_{15}H_{24}$	19.19	1.56
25	Benzene, 1,4-dimethoxy-2-methyl-5-isopropyl-	$C_{12}H_{18}0_2$	19.40	0.92
26	Cis-β-farnesene	$C_{15}H_{24}$	21.99	0.67
27	Spiro- [5.5] undec-2-ene, 3, 3, 7-trimethyl-11-methylene-, (-) -	$C_{15}H_{24}$	23.60	2.02
28	α-uurolen	$C_{15}H_{24}$	23.97	0.86
29	(z)-1-methyl-4-(6-methylhept-5-en-2-ylidene)cyclohexe-1-ene	$C_{15}H_{24}$	25.24	3.66
30	α-cuprenene	$C_{15}H_{24}$	25.80	2.64
31	(1S, 2E, 6E, 10R)-3, 7, 11, 11-tetram-ethylbicyclo [8.1.0] undeca-2, 6-diene	$C_{15}H_{24}$	26.14	0.45
32	β-bisabolene	$C_{15}H_{24}$	26.91	0.51
33	1-isopropyl-4, 7-dimelthyl-1, 2, 3, 5, 6，8a-hexahydronaphthalene	$C_{15}H_{24}$	28.60	0.25
34	(1R,2R,5S)-1, 8-dimethyl-4-(prop-1-en-2-yl) spiro [4.5] dec-7-ene	$C_{15}H_{24}$	29.43	0.96
35	Nerolidol 2	$C_{15}H_{26}O$	32.81	0.23
46	Epicubenol	$C_{15}H_{26}O$	36.72	0.61
37	Ylangenol	$C_{15}H_{24}O$	37.25	3.14
38	(1R, 2R, 4S, 6S, 7S, 8S)-8-isopropyl-1-methyl-3-methylenethylenetricycli [4.4.0.02.7] decan-4-ol	$C_{15}H_{24}O$	41.62	4.15
39	(1aR, 4aS, 8aS)-4a, 8, 8-trimethyl-11a, 4, 4a, 5, 6, 7,8-octahydrocyclopropa [d] naphthalene-2-carbaldehyde	$C_{15}H_{22}O$	42.41	3.38
40	Ylangenal	$C_{15}H_{22}O$	43.04	1.36

activities including antioxidant, antibacterial, and anticancer effect [31]. Until now, several studies reported the components of SCEO. However, there was variation in the types and yield [32, 33]. This was related to the growth environment of plants such as soil, climate, nutritional status, and extraction methods. The SCEO was considered a suitable source of terpenoids.

Antioxidant activity

Due to the complexity of the chemical components in plant extracts, it was reasonable to choose at least two methods for evaluating antioxidant activity to ensure authenticity of results, such as DPPH radical and $ABTS^+$ radical scavenging assays, as well as FRAP. The antioxidant activity of SCEO was compared between the hydrodistillation and UEAH methods. The results showed that the scavenging effect of DPPH radical using two different methods increased with concentration (Figure 4). The scavenging effect of SCEO extracted by UEAH

method demonstrated a higher inhibition percentage compared to hydrodistillation, and EC_{50} values were 0.939 ± 0.0060 mg/mL and 1.461 ± 0.026 mg/mL, respectively.

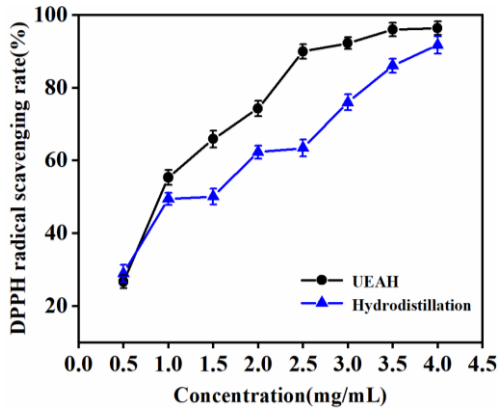

Figure 4. DPPH radical scavenging activity of SCEO by different extraction methods.

Recently, the $ABTS^+$ radical assay has been extensively utilized for assessing the antioxidant activity of plants and herbal extracts due to its rapid electron transfer. The result demonstrated that UEAH method exhibited a higher inhibition effect on the scavenging of $ABTS^+$ radical compared to hydrodistillation with the EC_{50} values of 0.260 ± 0.007 mg/mL and 0.305 ± 0.006 mg/mL, respectively (Figure 5). The scavenging effect showed minimal variation at a concentration of 1.0 mg/mL.

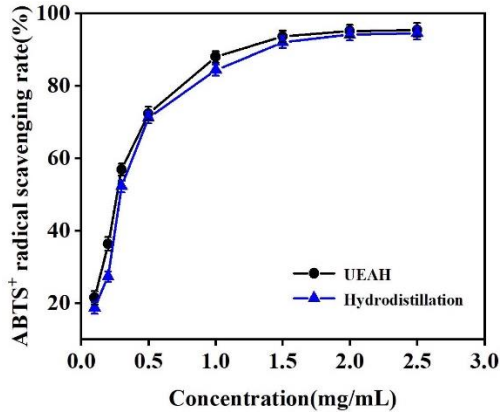

Figure 5. $ABTS^+$ radical scavenging activity of SCEO by different extraction methods.

The FRAP assay was widely employed in the analysis of food and health products and it was determined by the formation of blue-purple complex (ferrous ion and tripyridyltriazine). The iron reducing power of UEAH method (EC_{50} = 0.757 ± 0.031 mg/mL) was superior to hydrodistillation (EC_{50} = 1.459 ± 0.024 mg/mL) (Figure 6). The scavenging activity of free radicals and FRAP were related to the chemical components of SCEO. The types and content of dominant components obtained by UEAH in the SCEO might be higher than those obtained by hydrodistillation.

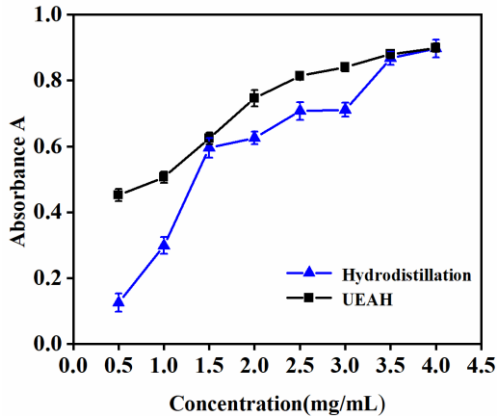

Figure 6. Ferric ion reducing antioxidant power of SCEO by different extraction methods.

Conclusion

The essential oil was extracted from *Schisandra chinensis* by ultrasound and enzyme assisted hydrodistillation (UEAH) in this study. Through single-factor experiments, it was found that time, pH, and temperature had significant effects on the extraction rate. The result of response surface methodology demonstrated that the optimal extraction conditions were 41.148 min, pH 4.58, and 56.248°C. The extraction rate under the optimal condition was 1.461%. Phytochemical analysis revealed that terpenoids accounted for the majority with copaene and γ-terpinene making up 28.83% and 8.62% of the content, respectively. The antioxidant experiments showed that the activity of essential

oil obtained using UEAH method was superior to that obtained through hydrodistillation, indicating that this extraction process could significantly improve the quality of essential oil. The UEAH method proposed in this study was considered as a simple and feasible extraction technology, offering an important perspective for the development and comprehensive utilization of *Schisandra chinensis* in the field of food and medicines.

Acknowledgements

This research was supported by Basic Scientific Research Project for Heilongjiang Provincial Colleges and Universities (Grant No. 2021-KYYWF-0588).

References

1. Li Z, He X, Liu F, Wang J, Feng J. 2018. A review of polysaccharides from *Schisandra chinensis* and *Schisandra sphenanthera*: Properties, functions and applications. Carbohydr Polym. 184(15):178-190.
2. Yang S, Yuan C. 2021. *Schisandra chinensis*: A comprehensive review on its phytochemicals and biological activities. Arab J Chem. 14(9):1-20.
3. Zhong S, Bai L, Liu X, Cai D, Yau L, Huang C, *et al*. 2021. Cough inhibition activity of *Schisandra chinensis* in Guinea pigs. J Med Food. 24(4):348-357.
4. Jung S, Moon H, Kim S, Quynh NTN, Yu J, Sandag Z, *et al*. 2019. Anticancer activity of gomisin J from *Schisandra chinensis* fruit. Oncol Rep. 41(1):711-717.
5. Li CL, Tsuang YH, Tsai TH. 2019. Neuroprotective effect of *Schisandra chinensis* on methyl-4-phenyl-1,2,3, 6-tetrahydropyridine-induced parkinsonian syndrome in C57BL/6 Mice. Nutrients. 11(7):1-17.
6. Tao X, Liang S, Che JY, Li H, Sun HX, Chen JG, *et al*. 2019. Antidiabetic activity of acidic polysaccharide from *Schisandra chinensis* in STZ-induced diabetic mice. Nat Prod Commun. 14(6):1-6.
7. Szopa A, Ekiert R, Ekiert H. 2017. Current knowledge of *Schisandra chinensis* (Turcz.) Baill. (Chinese magnolia vine) as a medicinal plant species: a review on the bioactive components, pharmacological properties, analytical and biotechnological studies. Phytochem Rev. 16(2):195-218.
8. Wang JW, Liang FY, Ouyang XS, Li PB, Pei Z, Su WW, *et al*. 2018. Evaluation of neuroactive effects of ethanol extract of *Schisandra chinensis*, *Schisandrin*, and *Schisandrin* B and determination of underlying mechanisms by zebrafish behavioral profiling. Chin J Nat Medicines. 16(12):916-925.
9. Chen X, Tang R, Liu T, Dai W, Liu Q, Gong G, *et al*. 2019. Physicochemical properties, antioxidant activity and immunological effects in vitro of polysaccharides from *Schisandra sphenanthera* and *Schisandra chinensis*. Int J Biol Macromol. 131(15):744-751.
10. Guo X, Liu L, Zhao W, Li X, Wang X, Ning A, *et al*. 2024. The protective effect of *Schisandra chinensis* (Turcz.) Baill. polysaccharide on DSS-induced ulcerative colitis in mice *via* the modulation of gut microbiota and inhibition of NF-κB activation. J Sci Food Agr. 104(1):196-206.
11. Wang X, Liu Y, Zhou S, Qin X, Gu W. 2017. Comparison on the compositions of essential oils from the seed and pulp of *Schisandra sphnanthera* Rehd. et Wils. J Essent Oil Bear PL. 20(4): 1066-1073.
12. Jin YP, Ai J, Qu Z, Cui L, Wang Y. 2019. Composition variation of essential oil from the stems of *Schisandra chinensis* with different fruit colors. J Essent Oil Bear PL. 22(6):1537-1543.
13. Xu M, Zhang X, Ren F, Yan T, Wu B, Bi K, *et al*. 2019. Essential oil of *Schisandra chinensis* ameliorates cognitive decline in mice by alleviating inflammation. Food Funct. 10(9):5827-5842.
14. Tan L, Yang Y, Peng J, Zhang Y, Wu B, He B, *et al*. 2022. *Schisandra chinensis* (Turcz.) Baill. essential oil exhibits antidepressant-like efects and against brain oxidative stress through Nrf2/HO-1 pathway activation. Metab Brain Dis. 37(6):2261-2275.
15. Zhao J, Ding K, Hou M, Li Y, Hou X, Dai W, *et al*. 2022. *Schisandra chinensis* essential oil attenuates acetaminophen-induced liver injury through alleviating oxidative stress and activating autophagy. Pharm Biol. 60(1):958-967.
16. Malaka MS, Naidoo K, Kabuba J. 2017. Extraction of *Siphonochilus aethiopicus* essential oil by steam distillation. Chem Eng Commun. 204(7):813-819.
17. Ara KM, Raofie F. 2016. Application of response surface methodology for the optimization of supercritical fluid extraction of essential oil from pomegranate (*Punica granatum* L) peel. J Food Sci Tech. 53(7):3113-3121.
18. Arce A, Marchiaro A, Rodríguez O, Soto A. 2006. Essential oil terpenless by extraction using organic solvents or ionic liquids. Aiche J. 52(6):2089-2097.
19. Drinić Z, Pljevljakušić D, Živković J, Bigović D, Šavikin K. 2020. Microwave-assisted extraction of *O. vulgare* L. spp. hirtum essential oil: comparison with conventional hydro-distillation. Food Bioprod Process. 120(3):158-165.
20. Wang CW, Zhang YY, Zhang X, Zheng KL, Cong Y, Yang L, *et al*. 2024. Enzyme-assisted extraction of essential oil from *Cinnamomum longepaniculatum* (Gamble) N. Chao ex H.W.Li and anxiolytic activity. Chem Pap. 78(4):4567-4582.
21. Rasouli SR, Ebrahimi SN, Rezadoost H. 2021. Simultaneous ultrasound-assisted hydrodistillation of essential oil from aerial parts of the *Satureja khuzistanica* Jamzad and its antibacterial activity. J Med Plants. 20(80):47-59.
22. Wang H, Tong Y, Li W, Zhang X, Gao X, Yong J, *et al*. 2017. Enhanced ultrasound-assisted enzymatic hydrolysis extraction of quinolizidine alkaloids from *Sophora alopecuroides* L. seeds. J Nat Med. 72(12):424-432.
23. Wei C, Xiao K, Li H, Qi Y, Zou Z, Liu Z. 2022. Optimization of ultrasound assisted aqueous enzymatic extraction of oil from

Cinnamomum camphora seeds. Lwt-Food Sci Technol. 164(15):1-11.

24. Vivek K, Mishra S, Pradhan RC. 2018. Optimization of ultrasound-assisted enzymatic extraction of Sohiong (*Prunus nepalensis*) juice. J Food Process Eng. 42(1):1-9.
25. Wang L, Chen Y, Song Y, Chen Y, Liu X. 2008. GC-MS of volatile components of *Schisandra chinensis* obtained by supercritical fluid and conventional extraction. J Sep Sci. 31(18):3238-3245.
26. Ma C, Liu T, Yang L, Zu Y, Chen X, Zhang L, *et al*. 2011. Ionic liquid-based microwave-assisted extraction of essential oil and biphenyl cyclooctene lignans from *Schisandra chinensis* Baill fruits. J Chromatogr A. 1218(48):8573-8580.
27. Chen X, Zhang Y, Zu Y, Fu Y, Wang W. 2011. Composition and biological activities of the essential oil from *Schisandra chinensis* obtained by solvent-free microwave extraction. LWT-Food Sci Technol. 44(10):2047-2052.
28. Blois MS. 1958. Antioxidant determinations by the use of a stable free radical. Nature. 181(4):1190-1200.
29. Kotora P, Šeršeň F, Filo J, Loos D, Gregáň J, Gregáň F. 2016. The scavenging of DPPH, galvinoxyl and ABTS radicals by imine analogs of resveratrol. Molecules. 21(2):1-13.
30. Siddiq M, Dolan KD, Perkins-Veazie P, Collins JK. 2018. Effect of pectinolytic and cellulytic enzymes on the physical, chemical, and antioxidant properties of blueberry (*Vaccinium corymbosum* L.) juice. Lwt-food Sci Technol. 92(6):127-132.
31. Yang W, Chen X, Li Y, Guo S, Wang Z, Yu X. 2020. Advances in pharmacological activities of terpenoids. Nat Prod Commun. 15(3):1-13.
32. Chen X, Zhang Y, Zu Y, Yang L. 2012. Chemical composition and antioxidant activity of the essential oil of *Schisandra chinensis* fruits. Nat Prod Res. 26(9):842-849.
33. Park MS, Park MS, Lee KG. 2020. Antioxidative activities of volatile and non-volatile extracts of *Schisandra chinensis* Baill fruit. Flavour Frag J. 35(4):435-442.