Optimization of Solvent-Free Microwave Extraction of Essential Oil from the Fruits of *Schisandra chinensis* and Its DPPH Radical Scavenging Activity

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Abstract：This study was undertaken to optimize the solvent-free microwave extraction conditions and DPPH radical-scavenging activity of essential oil from *Schisandra chinesis* fruits. The uniform design method was employed for process optimization. The optimal extraction conditions were determined as follows: extraction time, 50 min; microwave power, 800 W; and amount of water addition for pretreatment, 40%. Under these conditions, the extraction yield of essential oil was 0.92%. A total of 35 compounds were identified by GC-MS in the obtained essential oil with a total content of 91.06%, mostly consisting of ylangene (34.81%), β-himachalene (10.74%) and α-bergamotene (9.22%). The IC_{50} value of the essential oil against DPPH free radicals was determined as 3.01 mg/mL. In conclusion, solvent-free microwave extraction is a feasible method for essential oil extraction from *Schisandra chinensis* fruits.

Key words：*Schisandra chinesis*；essential oil；solvent-free microwave extraction；chemical composition；DPPH radical scavenging activity

五味子精油的无溶剂微波萃取工艺优化及DPPH自由基清除作用

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摘 要：为优化五味子精油的萃取工艺条件，采用无溶剂微波萃取技术萃取五味子精油，考察了3个变量(萃取时间，微波功率，预处理加水量)对精油得率的影响，并通过均匀设计法确定最佳萃取工艺条件；利用GC-MS对优化条件下得到的精油进行成分分析，通过DPPH法检测精油的自由基清除能力。结果表明：最佳的工艺条件为萃取时间50min、微波功率800W、预处理加水量40%，优化的精油得率为0.92%；精油的GC-MS分析共鉴定出35种成分，占精油总量的91.06%，依兰烯(34.81%)、β-雪松烯(10.74%)和α-佛手柑油烯(9.22%)为其中的3种主要成分；精油清除DPPH自由基的IC_{50}值为 3.01mg/mL。采用无溶剂微波萃取五味子精油工艺可行。

关键词：五味子；精油；无溶剂微波萃取；化学成分；DPPH自由基清除活性
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Essential oils are complex mixers comprising many volatile compounds derived chiefly from plants. In recent years, there has been a growing interest in using essential oils in aromatherapy, which claims that the specific essential oils have excellent bioactivities (antifungal, antioxidant, larvicidal, cytotoxic, apoptotic activity, and so on)[1-4]. Essential oils from many plants have antimicrobial and antioxidant properties which are used in food preservation, natural therapies, cosmetic and pharmaceutical industries.

The fruits of *Schisandra chinensis* (Turcz.) Baill. have been used as a tonic and sedative to treat chronic cough, spontaneous sweating, palpitation, and spermatorrhea in various prescriptions in China[5]. Isolation and identification of the components of *S. chinensis* have been extensively carried out since the 1950s[6]. Several active components in *S. chinensis* have been studied, including lignans[7], terpenoids[8] and polysaccharides[9]. Many studies have shown that the main bioactive components of *S. chinensis* are lignans[6-7].

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Furthermore, it has been suggested that the essential oil of *S. chinensis* possesses various bioactivities such as preventing cough, inhibiting plasminogen activation, and promoting DNA synthesis[10].

Solvent-free microwave extraction (SFME) is a combination of microwave heating and distillation to extract essential oils from plant materials. In SFME, there is no need to add solvent or water if fresh plant material is used and, if dry plant material is used, the sample is moistened by soaking in water. SFME has been used to the extraction of essential oils from aromatic herbs[11-12] and spices[13-15].

To the best of our knowledge, there is no information on the antioxidant capacity of the essential oil of *S. chinensis* using SFME. In the present study, use of SFME in the extraction of essential oil from *S. chinesis* was examined. A uniform design was developed to rank the importance of the three major factors affecting the SFME. The chemical composition of the essential oil resulting from the optimized conditions was analyzed and the antioxidant activity of the essential oil was evaluated by DPPH free radical-scavenging assay.

1 Materials and Methods

1.1 Materials and reagent

S. chinensis were purchased from Chinese Herb Store, Harbin, China. The plant material was identified by Prof. Nie Shaoquan. A voucher specimen (No. 037001001061002) was stored in the herbarium of the Key Laboratory of Forest Plant Ecology (Northeast Forestry University).

All solvents and chemicals were of analytical grade and obtained from local suppliers. DPPH, BHT and other chemical reagents were purchased from Sigma-Aldrich (St. Louis, MO).

1.2 Instruments

MAS-II Microwave synthesis reaction workstation was purchaseed from Sineo Microwave Chemistry Technology Company, Limited, Shanghai, China), 6890GC/MS from Agilent, USA, and UV-2550Spectrophotometer from Shimadzu, Japan.

1.3 Solvent-free microwave extraction

In a typical SFME procedure carried out at atmospheric pressure. 100 g plant material was moistened by soaking in water, and then placed in round-bottom flask (500 mL) and heated. A cooling system outside the microwave cavity continuously condensed the distillate. Condensed water was refluxed to the extraction vessel to provide uniform conditions of temperature and humidity for extraction. The essential oil was collected in amber-colored vials, essential oil was dehydrated with anhydrous sodium sulphate with the scale of 1 mL: 1.0 g, and then stored at 4 ℃ until analysis and testing.

A uniform design (UD) was used to investigate the optimal conditions of extraction of the essential oil from *S. chinensis*. Extraction was carried out with three factors and varies levels: extraction time (20, 30, 40, 50 min), microwave power (300, 400, 500, 600, 700, 800 W) and amount of water (40%, 60%, 80%, 100%, 120%, 140%; Table 1). The range of each factor was based on the results of preliminary experiments. The yield (%) of essential oil was the dependent variable.

1.4 Analysis of essential oil

The essential oil was analyzed using an Agilent 6890 gas chromatograph equipped with a flame ionization detector (FID) and DB-17MS capillary column (30 m × 0.25 mm; film thickness, 0.25 μm). Injector and detector temperatures were set at 220 ℃ and 290 ℃, respectively. The oven temperature of the gas chromatography (GC) machine was raised from 40 ℃ to 250 ℃ at a rate of 5 ℃/min. Helium was the carrier gas; at a flow rate of 1 mL/min. Diluted samples (1: 50 in ether, *V/V*) of 1.0 μL were injected manually and in splitless mode. Quantitative data were obtained electronically from FID area percentage data without the use of correction factors.

The analysis of the essential oil was undertaken under the same conditions with GC using an Agilent 6890 gas chromatograph equipped with an Agilent 5973 inert mass-selective detector in the electron impact mode (70 eV). Identification of the components was based on comparisons of their relative retention times and mass spectra with those obtained from standards in the NIST 02 mass spectral library. Alkanes (AccuStandard, Incorporated, New Haven, CT, USA) were used as reference points to calculate the relative retention indices (RRI).

1.5 DPPH radical scavenging activity

A modified DPPH assay[16] was used to measure the DPPH free radical-scavenging capacity of the essential oil of *S. chinensis*. A solution of DPPH in methanol (25 μg/mL) was prepared, and 1.8 mL of this solution was added to 0.2 mL solution of essential oil in methanol at different concentrations. The mixture was strongly shaken and maintained at room temperature for 30 min in the darkness. Then, the absorbance was measured at 517 nm in a spectrophotometer after 30 min. Radical-scavenging activity was calculated using the following equation:

$$\text{Scavenging effect /\%} = \frac{A_0 - A_t}{A_0} \times 100$$

Where A_0 is the absorbance of the control sample (without essential oil) and A_t is the absorbance in the presence of the sample (t=30 min). The sample concentration providing 50% inhibition (IC_{50}) was calculated by plotting inhibition percentages against sample concentrations.

1.6 Statistical analysis

Each of the measurements described above was carried out in triplicate. Results were expressed as mean ± standard deviations, and analyzed with Data Processing System (DPS Version 9.5, Hangzhou Reifeng Info-Technology Corp. Limited).

2 Results and Discussion

2.1 Optimization of extraction conditions

The Uniform design (UD) has been successfully used in various fields such as chemistry and chemical engineering, pharmaceutics, and survey design. The main advantage of UD is that it can be used for experiments in which the number of factors and levels of the factor are greater than traditional designs[17].

Table 1 Uniform design U_{12} ($4^1 \times 6^2$) and yield of the essential oil of *S. chinensis* fruits

No.	X_1 Extraction time/min	X_2 Microwave power/W	X_3 Amount of water/%	Yield/%
1	40	300	80	0.50
2	20	300	100	0.40
3	30	500	120	0.58
4	40	600	120	0.78
5	50	500	60	0.86
6	50	800	100	0.84
7	20	700	140	0.60
8	40	700	40	0.82
9	30	800	80	0.78
10	20	600	60	0.60
11	30	400	40	0.48
12	50	400	140	0.62

On the basis of preliminary experiments, the effects of some factors on extraction yield of essential oil, such as extraction time, power and amount of water were considered, and the main factors and the level setting values are shown in Table 1. The yield was the mass of essential oil extracted relative to the mass of *S. chinensis* powder. A regression analysis was carried out to fit mathematical models to the experimental data aiming at an optimal region for the studied. The second-order polynomial stepwise regression analysis model, which is an empirical relationship between the yield and the test variable in coded unit as given in Eq. (1), can describe the predicted model.

$$Y = -0.994049571 + 0.015728040494X_1 + 0.0027750172366X_2 + 0.009206264350X_3 - 0.0000015518707483X_2^2 - 0.000028443877551X_3^2 - 0.0000030952380952X_1X_2 - 0.00006871983022X_1X_3 - 0.000004073992987X_2X_3 \quad (1)$$

Eq. (1) indicates that the extraction efficiency was significantly influenced by all the three parameters investigated, and the influences of the parameters were interactive. The multiple coefficients of correlation R=0.985066 indicated a close agreement between experimental and predicted values of the essential oil yield. The coefficient of determination (R^2) of the predicted model was 0.970355, suggesting a good fit, and the predicted model seemed to reasonably represent the observed values. The optimal values of the variables given by the software are the following: extraction time 50 min, power 786.2130 W and amount of water 43.1047%, the estimated 'maximum extraction yield' based on this model is 0.9510%.

As expected, the extraction yield of the essential oil increases with the three factors. Indeed, as it appeared in the preliminary study, SFME extraction can be continued until no more essential oil is extracted, as in hydro-distillation (HD). However, extraction time of SFME should be lower than that of HD to be interesting in terms of extraction efficiency. The irradiation power determines the rate of evaporation of water or the mixture of water and essential oil during SFME. The greater the rate of evaporation, the greater the yield of essential oil extracted. The humidity level of the matrix under microwave heating is crucial as it was emphasized by the preliminary study. Water absorbs microwaves, and then heats, allows giving an extraction temperature close to 100 ℃. This heated in situ water creates areas of compression in the powder, surrounded by areas of lower pressure, making the glands and oleiferous receptacles burst, then the oil flows to the exterior.

2.2 Chemical composition of the essential oil

The yellowish essential oil of *S. chinensis* was obtained by optimized SFME in the yield of 0.92%. The components of the essential oil were analyzed by gas chromatography-mass spectrometry (GC-MS). Thirty-five components were identified, representing 91.06% of the total oil. Their relative retention indices and percentages were summarized in Table

2. The essential oil samples were rich in sesquiterpenes. Ylangene (34.81%), β-himachalene (10.74%) and α-bergamotene (9.22%) were the main components, comprising the 54.77% of the essential oil.

Table 2 Chemical composition of the essential oil of S. chinensis fruits obtained by SFME

No.	Compound[a]	Relative retention index[b]	Relative contents/%
1	terpinene	1151	0.35
2	methylthymol	1387	0.51
3	bornyl acetate	1425	1.14
4	cycloisosativene	1440	0.33
5	ylangene	1446	34.81
6	β-bourbonene	1470	0.24
7	elemene	1481	0.24
8	terpinolene	1494	0.13
9	α-santalene	1498	0.18
10	cycloisolongifolene	1523	0.79
11	β-sesquiphellandrene	1547	0.57
12	caryophyllene	1566	0.13
13	γ-cadinene	1572	0.23
14	isocaryophillene	1580	0.18
15	α-himachalene	1583	0.61
16	α-bergamotene	1588	9.22
17	epizonarene	1592	0.30
18	elemene	1597	0.93
19	α- muurolene	1599	0.82
20	β-chamigrene	1613	5.91
21	β-himachalene	1634	10.74
22	α-chamigrene	1640	1.29
23	α-acoradiene	1645	3.06
24	δ-cadinene	1654	0.31
25	isoledene	1662	0.45
26	cuparene	1675	0.70
27	himachalene	1680	1.12
28	nerolidol	1696	0.10
29	eudesma-4(14),11-diene	1735	0.25
30	copaene	1834	4.46
31	γ-selinene	1850	0.13
32	α-longipinene	1853	0.59
33	humulen	1867	0.23
34	cis-calamenene	1939	6.79
35	δ-cuparenol	2092	3.22
	Total		91.06

Note: a. Compounds listed in order of elution from DB-17MS capillary column; b. Relative retention indices to $C_{11}-C_{21}$ *n*-alkanes on DB-17MS capillary column.

The composition of the essential oil of *S. chinensis* obtained by hydrodistillation has been published[18-19]. According to Li et al[18], α -farnesene (14.37%), copaene (11.93%) and patchulane (8.25%) constituted the major components of the essential oil of *S. chinensis*. While, according to Zhu et al[19], the ylangene content is the highest, but only at 14.34%. Comparative study of the chemical composition of the essential oil from species of the genus Schizandra is poor because the constituents of the essential oils of plants may vary according to geographical, climatic, seasonal, and experimental conditions.

2.3 DPPH radical scavenging assay

Several reports have published on the biological activities of the species of Schisandra genus[9-10]. However, we could not find report dealing with the antioxidant activity of *S. chinensis* essential oil. In the present study, the free radical-scavenging capacity of the essential oil was evaluated by the DPPH assay (Figure 1). There was positive correlation between DPPH radical scavenging-activity and concentration of the essential oil. A lower IC_{50} value and greater DPPH radical scavenging percents indicate higher antioxidant activity. The IC_{50} of the essential oil was (3.01±0.15) mg/mL, which was greater than that of the synthetic antioxidant BHT (IC_{50}=0.43 mg/mL±0.05 mg/mL). Antioxidant activity of the essential oil at 4.5 mg/mL was similar to BHT at 0.5 mg/mL. The biological activities of essential oils usually depend on their major components. Researchers studying the antioxidant activity of the chemical components of essential oils have shown that monoterpenes have a higher antioxidant effect[20]. Moreover, some essential oils rich in non-phenolic compounds also have antioxidant potential[21]. The major components of *S. chinensis* essential oil were determined to be sesquiterpenes (Table 2). Therefore, we think the lack of monoterpenes in the essential oil of *S. chinensis* is one of the possible reasons for its weak antioxidant activity.

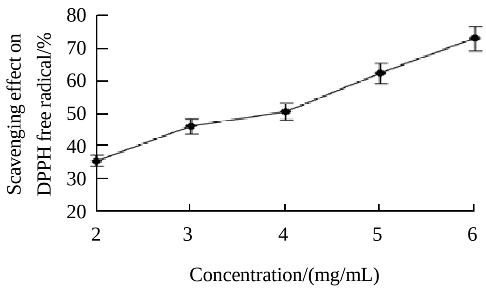

Fig.1 Free radical scavenging properties of the essential oil of *S. chinensis* fruits

3 Conclusions

The present study supports the idea that SFME could be a reliable method for the extraction of the essential oil from *S. chinensis* fruits, the yield was 0.92% under the optimum conditions. The compositions of the essential oil obtained by the optimized SFME indicated that the

S. chinensis essential oil had a high content of sesquiterpenes, the main components were langene (34.81%), β-himachalene (10.74%) and α-bergamotene (9.22%) representing 54.77% of the oil. The essential oil exhibited weaker antioxidant ability (IC_{50} 3.01 mg/mL) compared with those of BHT. However, *S. chinensis* fruits have been used in foods and medicines with a long history in China; further investigation to evaluate the practical effectiveness of the essential oil in particular antimicrobial activity is needed.

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