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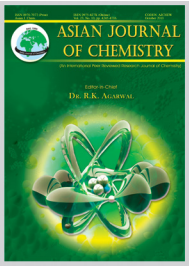

Characterization of the Essential Oil in Leaves of *Chimonanthus praecox* var. *concolor*

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Wintersweet [*Chimonanthus praecox* (L.) Link] belonging to the Calycanthaceae family is a famous traditional fragrant flower plant with high ornamental value. The extraction conditions of hydrodistillation were optimized for the essential oil in leaves of *C. praecox* var. *concolor* using orthogonal design. The best extraction conditions were established as comminution degree of wintersweet leaves was 0.9 mm, the material-liquid ratio for 5-fold water, the maceration and distillation times for 2 h and 8.5 h, respectively. Twenty-three components in the essential oils were identified by gas chromatography-mass spectrometry (GC-MS). The main chemical compounds of the essential oil were bornyl acetate (20.98 %), caryophylene oxide (18.49 %), borneol (4.36 %), (+)-δ-cadinene (4.95 %), elemol (4.24 %), juniper camphor (4.21 %) and phytol (4.29 %), which might be associated with the fragrancy.

Key Words: *Chimonanthus praecox* var. *concolor*, Essential oil, Hydrodistillation, Orthogonal test, Chromatography-mass spectrometry.

INTRODUCTION

Chimonanthus praecox (L.) link, namely wintersweet, belonging to the Calycanthaceae family is a deciduous shrub native to China. It is a famous traditional fragrant flower plant with high ornamental value in China. Wintersweet roots, stems, leaves, buds, fruit and flowers can be used as medicine, with the good pharmacological effects of detumescence and promoting blood circulation *etc.*[1].

C. praecox var. *concolor* has pure yellow flowers with thick flavour, being an important fragrant plant. Mariko *et al.*[2] reported flowers and leaves of *Chimonanthus* plants generally have aromatic oil. In recent years, several studies have been performed on chemical composition of essential oils in leaves of several *Chimonanthus* species[3-7]. However, less information has been reported on essential oil in leaves of *C. praecox* var. *concolor*.

Hydrodistillation, organic solvent and supercritical fluid extraction methods have been mainly used for extracting plant essential oil[8,9]. Of which, hydrodistillation method has been widely used for extracting essential oil from plants due to the superiorities like just simple equipment, easy operation, lower cost and pure products. However, the best conditions for extracting essential oil from wintersweet leaves through hydrodistillation, has less been reported. In this paper, the objectives are to establish the best hydrodistillation extraction conditions for getting higher essential oil yield, then to investigate the essential oil characteristics in leaves of *C. praecox* var. *concolor* by the optimized hydrodistillation extraction conditions and chromatography-mass spectrometry (GC-MS).

EXPERIMENTAL

Plant materials: The wintersweets of *Chimonanthus praecox* var. *concolor* were grown at the Taipingzhen in Wanyuan City of Sichuan, China. The green and fresh leaves on middle-upper part of perennial branches of the plants were collected by hands in July 2009. And, the leaves were dried in the shade.

The essential oil in wintersweet leaves was extracted as the method in Pharmacopoeia of the People's Republic of China[10]. According to the designs of single factor and orthogonal tests, the essential oil of wintersweet leaves was studied using the optimized hydrodistillation extraction conditions. To detect the components of essential oil, GC-MS (gas chromatography-mass spectrometry) analysis was performed.

The obtained essential oil was weighed as the above method and the extraction rate was evaluated as the following equation. And, the variance analysis was carried out by the DPS v6.55 procedure.

$$\text{Essential oil extraction rate (\%)} = \frac{\text{Weight of the essential oil extracted}}{\text{Weight of the leaves tested}} \times 100$$

Essential oil extraction

Designs of single factor and orthogonal tests: The essential oil extraction rate was taken as the index and the four parameters like the leaf comminution degree, material-liquid ratio, immersion and distillation times as affecting factors with different treatment levels in single factor tests. The leaves were ground for 5 mm, 0.9 mm (20 mesh powder), 0.3 mm (60 mesh powder), respectively. The distillation water for 4-fold, 5-fold, 6-fold and 7-fold volume of the plant materials by the material-liquid ratio, was respectively infused. The leaf powders were macerated for 0, 0.5, 1, 1.5, 2, 4 and 6 h, respectively. The hydrodistillation was carried out for 2.5, 4.5, 6.5, 8.5, 10.5 and 12.5 h, respectively.

Because the extraction efficiency of essential oil might be varied with the different combinations among both factors and their levels, the orthogonal test based on the results of single factor tests was designed (Tables 1 and 2) in order to probe the optimal extraction conditions of hydrodistillation.

Gas chromatography-mass spectrometry analysis: To detect the components of essential oil, gas chromatography-mass spectrometry (GC-MS) analysis was performed on a Agilent 6890 gas chromatography instrument coupled to a 5973 N mass spectrometer (Agilent company), equipped with a DB-1 capillary column (31.7 m × 0.25 mm I.D., film thickness 0.25 μm). The column was maintained at 75 ºC for 1 min and programmed at 2 ºC/min from 75 ºC to 110 ºC, then at 3 ºC/min to 130 ºC, at 1 ºC/min to 150 ºC, at 15 ºC/min to 220 ºC, at 5 ºC/min to 240 ºC, held for 5 min. The temperature of the injection port and interface was set at 250 ºC. Helium was used as the carrier gas with a flow rate of 1 mL/min. One microlitre of the sample was injected in the 30:1 split ratio. The mass spectrometer was operated under electron impact (EI) mode at ionization energy of 70 eV and mass range from 50 to 550 amu, the scan rate was 5 scan/s. The ionization source temperature was 230 ºC. The analytes were identified using the NIST Mass Spectral Database. The relative responses of individual components are expressed as percent peak area relative to total peak area.

RESULTS AND DISCUSSION

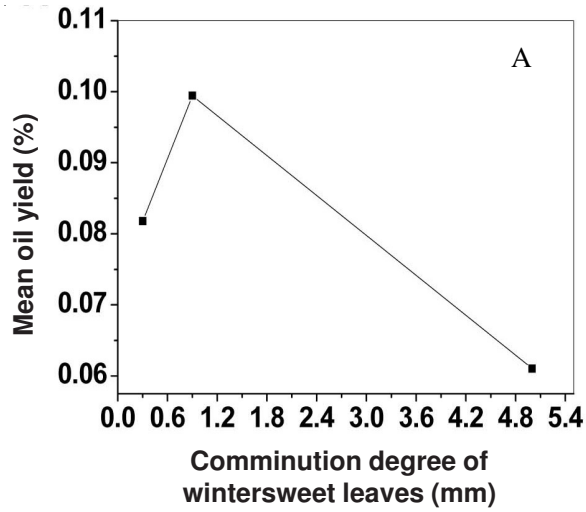
Essential oil extraction: In the analyses of single factor tests, the efficiencies of comminution degree, material-liquid ratios, time of immersion and distillation of wintersweet leaves for extraction essential oil, were respectively assessed (Fig. 1). The essential oil extraction rate was varied with the ground degrees of leaves (Fig. 1A). Of which, the comminuted leaves for 0.9 mm produced the highest essential oil extraction rate, accounting for 0.0995 %. And, the essential oil yield of the treatment for 5.0 mm, only for 0.0610 %, was lower than that for 0.3 mm for 0.0818 %. Apparently, the treatment for 0.9 mm, showed the best efficiency for essential oil extraction. It is possibly resulted from that the too small leaf powders easily cause many big bubbles, which easily makes them to be spilled and severe pasting which makes some of them to be lost, with the solution boiling, all of which might lead to the collection of volatile oil being reduced[11,12]. By contraries, the too big leaf shatters might be difficultly fully soaked, which easily makes the cells difficultly release the entocytes, resulting in the very low essential oil rate.

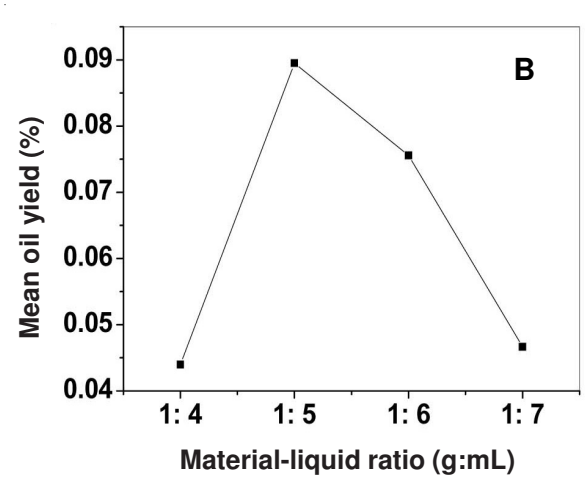
Different essential oil yields were obtained from the four treatments of material-liquid ratio (Fig. 1B). Of which, the treatment with 5-fold volume water produced the highest essential oil yield for 0.0895 %. When the water was added over 5-fold volume of the leaf materials, the extraction rate of essential oil showed a downward trend. It might be associated with these factors that excessive volume water easily results in explosive boiling and, too little water can not fully soak the extracted leaf materials.

The extraction rate of essential oil increased with the increase of soaking time over one hour and reached the quite high value for 0.0785 % for 2 h, but later, was less added (Fig. 1C). Because soaking can make plant cell gap larger, tissue cell fuller and intracellular fluid dynamics stronger, resulting in accelerating the exchanges of both matter and energy, which is conducive to the extraction of essential oil[12], the leaf organizations of immersion treatment for 2 h might be fully inflated and therefore release more essential oil molecule.

The extraction rate of essential oil also increased with the increase of distillation time and reached the high value for 0.1156 % for 8.5 h. Later, the essential oil yield was less increased, being relatively stable with distillation time prolonging (Fig. 1D). This shows that a majority of essential oil has been fully distilled when the time are for 8.5 h.

In the analysis of orthogonal test, three levels of each factor were selected on the basis of preliminary optimization





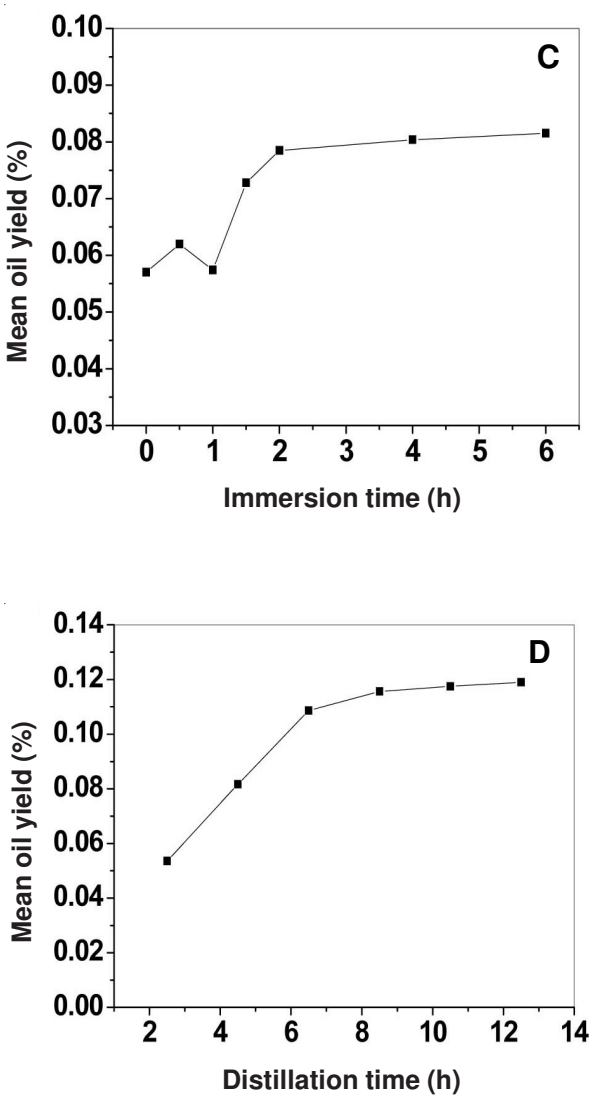


Fig. 1. Effect of the four factors comminution degree, material-liquid ratio, immersion and distillation times of wintersweet leaves on the extraction rate of essential oil. The figures A, B, C and D indicate the results of single factor tests like comminution degree (mm), material-liquid ratio (g:mL), immersion time (h) and distillation time (h) of wintersweet leaves, respectively

results of single-factor test. The orthogonal test of four factors combined with three levels was designed to optimize the steam distillation conditions for extracting essential oil from wintersweet leaves (Table-1).

TABLE-1
FACTORS AND LEVELS OF ORTHOGONAL TEST

A Comminution degree (mm)	B Material-liquid ratio (g:mL)	C Immersion time (h)	D Distillation time (h)
5.0	1:4	1.5	6.5
0.9	1:5	2.0	8.5
0.3	1:6	2.5	10.5

The range analysis results of orthogonal test indicated that for the extraction efficiency, the material-liquid ratio was the most important factor with the highest R value at 0.009383 %, followed by the comminution degree and the immersion time with the higher R values at 0.008688 % and 0.008162 %, respectively and the distillation time with the lowest R value at 0.002386 % (Table-2). All the four analyzed factors possessed the highest essential oil extraction rate at the level 2 with the highest K_2 values of 0.070974, 0.069777, 0.069593 and 0.066953 %, respectively, compared with the other two levels, indicating $A_2B_2C_2D_2$ were the most suitable conditions for extracting essential oil from wintersweet leaves. And, the variance analysis revealed that there were significant differences among the three levels of the two factors like the material-liquid ratio and the comminution degree ($df = 2$, $P < 0.01$) and of the factor like the immersion time ($df = 2$, $P < 0.05$) (Table-3). So, the optimum conditions of essential oil extraction were the leaf comminution degree for 0.9 mm, the material-liquid ratio for 5-fold volume water, the immersion and distillation times for 2 and 8.5 h, respectively.

In order to validate the optimum conditions, a sample made under these optimum conditions was determined. The result indicated that the extraction rate under the optimum conditions was higher than those under any other group in the orthogonal experiment, account for 0.121000 % (Tables 2 and 4).

GC-MS analysis: A total of 23 compounds were identified by GC-MS from the essential oil in leaves of *C. praecox* var.

TABLE-2
RESULTS OF L_9 (3^4) ORTHOGONAL TEST

Test trial	Column no.				Extraction rate (%)		
	A[a]	B[a]	C[a]	D[a]	X^1	X^2	$\overline{X}$
1	1[b]	1	1	1	0.078723	0.072355	0.075539
2	1	2	2	2	0.118497	0.092373	0.105435
3	1	3	3	3	0.105888	0.092744	0.099316
4	2[b]	1	2	3	0.112156	0.096168	0.104162
5	2	2	3	1	0.125496	0.097386	0.111441
6	2	3	1	2	0.117203	0.090360	0.103782
7	3[b]	1	3	2	0.098045	0.086098	0.092072
8	3	2	1	3	0.105792	0.088447	0.097120
9	3	3	2	1	0.111056	0.096084	0.103570
K_1[c]	0.062287	0.060394	0.061431	0.064567			
K_2[c]	0.070974	0.069777	0.069593	0.066953			
K_3[c]	0.065058	0.068148	0.067295	0.066799			
R[c]	0.008688	0.009383	0.008162	0.002386			

[a]The four tested factors; [b]The three tested levels for each factor; [c]Average response for each level; [1,2]The first and second time's extraction, respectively

TABLE-5
CHEMICAL COMPOSITION OF THE ESSENTIAL OIL OF *C. praecox* var. *concolor* LEAVES

No.	R.T. (min)	Compound	m.f.	m.w.	Relative content (%)
1	4.93	Camphene	$C_{10}H_{16}$	136	0.95
2	11.61	Borneol	$C_{10}H_{18}O$	154	4.36
3	17.65	Bornyl acetate	$C_{12}H_{20}O_2$	196	20.98
4	23.52	10-(acetylmethyl)-(+)-3-Carene	$C_{13}H_{20}O$	192	2.24
5	24.49	β-Elemene	$C_{15}H_{24}$	204	1.89
6	25.84	β -Caryophyllene	$C_{15}H_{24}$	204	2.10
7	29.00	β-Cubebene	$C_{15}H_{24}$	204	2.44
8	29.23	β-Selinene	$C_{15}H_{24}$	204	2.66
9	30.59	α-Muurolene	$C_{15}H_{24}$	204	1.87
10	31.56	(+)-delat-Cadinene	$C_{15}H_{24}$	204	4.95
11	32.68	Elemol	$C_{15}H_{26}O$	222	4.24
12	33.44	γ-Elemene	$C_{15}H_{24}$	204	1.78
13	34.27	(-)-Spathulenol	$C_{15}H_{24}O$	220	7.17
14	34.57	Caryophyllene oxide	$C_{15}H_{24}O$	220	18.49
15	36.24	Isoaromadendrene epoxide	$C_{15}H_{24}O$	220	2.40
16	37.78	γ-Himachalene	$C_{15}H_{24}$	204	1.34
17	38.75	Tau-Cadinol	$C_{15}H_{26}O$	222	1.83
18	39.10	β-Eudesmol	$C_{15}H_{26}O$	222	1.53
19	39.30	Juniper camphor	$C_{15}H_{26}O$	222	4.21
20	40.77	Ledene oxide-(II)	$C_{15}H_{24}O$	220	2.29
21	53.11	6,10,14-trimethyl-2-Pentadecanone	$C_{18}H_{36}O$	268	3.93
22	55.12	*n*-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	2.07
23	57.44	Phytol	$C_{20}H_{40}O$	296	4.29

TABLE-3
ANALYSIS OF VARIANCE

Factors	SS	Df	F	P	Significance
A	0.000532	2	9.340009	< 0.01	**
B	0.000679	2	11.921895	< 0.01	**
C	0.000478	2	8.400797	< 0.05	*
D	4.82E-05	2	0.846024	> 0.05	ns
Error	0.000228	8			

$F_{0.01}(2,8) = 4.46$, $F_{0.05}(2,8) = 8.65$

TABLE-4
EXTRACTION RATE OF VERIFICATION TEST

Repeat	X^1	X^2	$\bar{x}$	Standard error
Extraction rate (%)	0.120667	0.121333	0.121000	0.000333

[1,2]The first and second time's extraction, respectively

concolor, representing 100 % of the total peak area. The main components were bornyl acetate (20.98 %), caryophylene oxide (18.49 %), borneol (4.36 %), (+)-delat-cadinene (4.95 %), elemol (4.24 %), juniper camphor (4.21 %) and phytol (4.29 %). Many components of the essential oil were oxygenated drivatives of terpenes, which might have close link to its fragrance.

Conclusion

Steam distillation is an economic, convenient, efficient method for extracting essential oil. Especially, it corresponds to actual needs and environmental regulations. In this study, the optimum conditions of extracting essential oil from wintersweet leaves were set up using both single-factor and $L_9(3^4)$ orthogonal tests, which are the leaf comminution degree for 0.9 mm, the material-liquid ratio for 5-fold volume water, the material immersion and distillation times for 2 and 8.5 h, respectively. The essential oil was obtained by the optimized hydrodistillation extraction conditions from the leaves of *C. praecox* var. *concolor* and was characterized by GC-MS, which might be beneficial to better understand the aromatic properties of *C. praecox* var. *concolor*.

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