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Comparative analysis of essential oils from the leaves of wild and domestic *Chimonanthus praecox*

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Characteristics of leaf essential oil extracted by hydrodistillation and analyzed by gas chromatography-mass spectrometry (GC-MS) from wild and domestication *Chimonanthus praecox* from Wanyuan City of Sichuan, China, were investigated. Their leaves had twenty-two common essential oil components containing the mains bornyl acetate, caryophyllene oxide and (-)-spathulenol. Their oils were characterized by sesquiterpenes and monoterpenes, especially, the oxygenated. However, the wild plants possessed higher essential oil content and more abundant chemical components than the domestication variety "Suxinmei". The wild plants possessed sixty-four special components such as o-cymene, eucalyptol, γ -terpinene, linalool, etc, and most of them have enjoyable flavor and curative effect. But, "Suxinmei" was only with the three special components α -muurolene, γ -elemene and γ -himachalene, and without aromatic compounds. These results suggested that partial genetic materials controlling some especial nature products might have been changed or lost in the processes of evolution and domestication from wild to cultivar. It is of importance to powerfully protect and rationally utilize wild *C. praecox* germplasm resources.

Key words: *Chimonanthus praecox*, germplasm resources, leaf, hydrodistillation, GC-MS, essential oil, yield, chemical component.

INTRODUCTION

Chimonanthus praecox belonging to the Calycanthaceae family is a deciduous shrub native to China, which has survived from the tertiary period. It is a famous traditional fragrant flower plant with high ornamental value in China. In recent years, the domestication variety "Suxinmei" from *C. praecox* as a kind of rare landscape plants has been widely introduced and cultivated in other countries like North America, Europe, Japan, North Korea, etc (Ming and Liao, 2004). For largely propagating the ornamental variety "Suxinmei" to satisfy the bigger and bigger ornamental markets, innumerable wild *C. praecox* plants were dug as the grafting rootstock. And, for a long

time, wild *C. praecox* plants have been considered as worthless shaw, and felled as firewoods and fences. Additionally, wild *C. praecox* habitats have been increasingly destroyed because of various mining, constructing, etc. These have resulted in the wild resources in some regions being in severe danger, even in deracination (Wu et al., 2009; Cheng et al., 2007). The existing natural populations of *C. praecox* distributed in a very small minority of areas including the eastern of Sichuan, the northeast of Chongqing, the northwestern of Hunan, and the northwest of Hubei in China (Li and Li, 2000). Of which, Wanyuan City of Sichuan possessed more abundant wild resources than others (Wu et al., 2009; Zuo et al., 2009).

Chimonanthus plants are also traditional Chinese herbal medicine for the treatment of colds, analgesic, coughs, asthma and other disorders (Xiao, 2001). In

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recent years, research and development of essential oil products of *Chimonanthus* plants have been more and more attended, due to excellent aroma (Ming and Liao, 2004).

And, some data on essential oils in leaves from these species like *C. nitens* (Zhan and Xu, 2006; Xu et al., 2006; Cao et al., 2008; Shu et al., 2010), and *C. zhejiangensis* (Ou and Mai, 2010), as well as in flowers (Jing et al., 2005; Farsam et al., 2007; Li et al., 2008; Si et al., 2010; Zhao et al., 2010) and seeds (Li and Zhang 2006) from this species *C. praecox* have been provided. The previously reported *C. praecox* materials are all from domestication varieties like “Suxinmei”, “Qingkoumei”, etc. And, the essential oils from leaves of *C. praecox* including both the wild and cultivated have not been reported.

Large numbers of researches showed that both contents and chemical constituents of essential oil in plants are varied with many biologic and abiological factors such as plant species, variety, cultivar, organ, tissue, and climate, environment, geographical origin, etc (Zhang et al., 2002; Zeng et al., 2003; He et al., 2006; Wang et al., 2008; Wang et al., 2009; Huo et al., 2010). And, there were also significant differences between wild and domestication plants (Wu et al., 2009; Yu et al., 2009; Zhang et al., 2009; He et al., 2010). In this study, essential oil in the leaves of *C. praecox* from a natural wild population in Wanyuan City of Sichuan, China, was firstly investigated and compared with that of the domestication variety “Suxinmei”, for better understanding the aromatic and medical properties, as well as for providing the scientific groundwork of powerful protection and sustainable utilization of wild *C. praecox* germplasm resources.

MATERIALS AND METHODS

Plant materials

Two individual wild *C. praecox* plants W1 and W2 from a natural population, which are with red or purple speckles on the inside petals, and one “Suxinmei” plant C1 as the control with pure yellow flowers, which is a domesticated variety derived from natural mutations of wild *C. praecox*, were randomly selected at the Taipingzhen in Wanyuan City of Sichuan, China. The green and fresh leaves on middle-upper part of perennial branches were collected by hands, respectively, in July 2009. The leaves were dried in the shade and pulverized into powder for 20 meshes.

Essential oil extraction

According to the method in Pharmacopoeia of the People's Republic of China (The State Pharmacopoeia Commission of China, 2005), and as the optimum extraction conditions in our previous research (Wu et al., 2011), the leaf powders for 100 g were macerated in 5-fold volume water by the materials-liquid ratio for 2 h and subjected to hydrodistillation for 8.5 h, and then the essential oils were obtained. The essential oils were collected in EP (eppendorf) tubes and stored in ultra low temperature freezer at -80°C until chemically analyzed.

Gas chromatography-mass spectrometry

To detect the components of essential oil, GC-MS (gas chromatography-mass spectrometry) analysis was performed on a Agilent 6890 gas chromatography instrument coupled to a 5973N 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°C for 1 min, and programmed at 2°C/min from 75°C to 110°C, then at 3°C/min to 130°C, at 1°C/min to 150°C, at 15°C/min to 220°C, at 5°C/min to 240°C, held for 5 min.

The temperature of the injection port and interface was set at 250°C. Helium was used as the carrier gas with a flow rate of 1 ml/min. 1 μl 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°C. The chemical components of essential oil were identified using the NIST Mass Spectral Database. The relative response of individual component was expressed as percent peak area relative to total peak area.

RESULTS

Essential oil extraction

The extracts obtained from the leaves of the wild and the variety “Suxinmei” plants of *C. praecox* were pale-yellow oils with an aromatic-spicy odour. The yields of essential oil in leaves of wild plants W1 and W2 were different, accounting for 0.0999% and 0.0922%, respectively, which were higher than that of “Suxinmei” for 0.0842% (Figure 1).

Chemical composition of the essential oil

A total of forty-six and fifty-nine components were identified by GC-MS from the oils in leaves of the wild plants W1 and W2, respectively, while that of “Suxinmei” C1 only for twenty-five types (Table 1). Of which, twenty-two like borneol, terpinen-4-ol, bornyl acetate, damascenone, 10-(acetylmethyl)-(+)-3-carene, β-elemene, β-caryophyllene, α-guaiene, etc were detected in both the wild and control plants. The wild plants contained the sixty-four special components such as o-cymene, eucalyptol, γ-terpinene, linalool, (1s)-(-)-camphor, trans-pinocarveol, (s)-cis-verbenol, and so on, while “Suxinmei” only had the three special components α-murolene, γ-elemene and γ-himachalene.

The wild and “Suxinmei” plants had similar major components in their leaf essential oils. The preponderant components in the two wild W1 and W2 plants were caryophyllene oxide (10.51% and 11.98%), (-)-spathulenol (15.53% and 10.81%), bornyl acetate (15.90% and 17.44%), respectively. And, “Suxinmei” C1 had the alike main components like bornyl acetate (35.31%), caryophyllene oxide (11.26%) and (-)-spathulenol (3.55%). The amount of these components was over half of the oils.

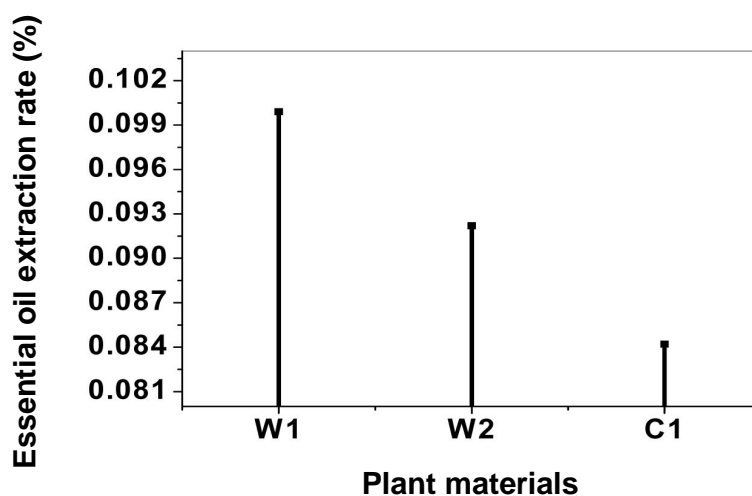


Figure 1. Comparison of essential oil yield from wild and domestication *C. praecox* leaves.

Table 1. Chemical composition of the leaf essential oil from wild and domestication *C. praecox*.

S/N	R.T (min)	Compound	Formula	Mw	Relative content (%)		
					W1	W2	C1
1	4.49	2-Thujene	C ₁₀ H ₁₆	136		0.24	
2	4.66	alpha-Pinene	C ₁₀ H ₁₆	136		0.99	
3	4.94	Camphene	C ₁₀ H ₁₆	136		1.70	
4	5.41	Sabinene	C ₁₀ H ₁₆	136		1.04	
5	5.53	(1S)-(1)-beta-Pinene	C ₁₀ H ₁₆	136		0.76	
6	6.53	alpha-Terpinene	C ₁₀ H ₁₆	136		0.29	
7	6.60	O-cymene	C ₁₀ H ₁₄	134	0.34	1.65	
8	6.86	Eucalyptol	C ₁₀ H ₁₈ O	154		0.69	
9	7.78	gamma-Terpinene	C ₁₀ H ₁₆	136		0.60	
10	9.03	Linalool	C ₁₀ H ₁₈ O	154	0.66	0.64	
11	10.37	(1S)-(-)-Camphor	C ₁₀ H ₁₆ O	152	0.34	0.26	
12	10.52	trans-Pinocarveol	C ₁₀ H ₁₆ O	152	0.41	0.56	
13	11.51	(S)-cis-Verbenol	C ₁₀ H ₁₆ O	152	0.36	0.44	
14	11.62	Borneol	C ₁₀ H ₁₈ O	154	4.27	3.23	5.75
15	12.22	Terpinen-4-ol	C ₁₀ H ₁₈ O	154	2.49	1.65	1.11
16	12.52	(-)-Myrtenal	C ₁₀ H ₁₄ O	150		0.36	
17	12.72	alpha-Terpineol	C ₁₀ H ₁₈ O	154	0.14	0.27	
18	13.01	Myrtenol	C ₁₀ H ₁₆ O	152		0.30	
19	14.57	4-(1-methylethyl)-Benzaldehyd	C ₁₀ H ₁₂ O	148	0.39	0.35	
20	15.97	Nerol	C ₁₀ H ₁₈ O	154	0.51		
21	17.66	Bornyl acetate	C ₁₂ H ₂₀ O ₂	196	15.90	17.44	35.31
22	19.58	p-Mentha-1,4-dien-7-ol	C ₁₀ H ₁₆ O	152		0.44	
23	23.07	Damascenone	C ₁₀ H ₁₈ O	190		0.71	
24	23.53	10-(acetylmethyl)-(+)-3-Carene	C ₁₃ H ₂₀ O	192	0.69	1.75	1.21
25	23.72	Copaene	C ₁₅ H ₂₄	204	1.53	1.30	3.00
26	24.08	beta-Bourbonene	C ₁₅ H ₂₄	204	1.09	0.77	
27	24.50	beta-Elemene	C ₁₅ H ₂₄	204	0.43	3.05	
28	25.03	9,10-dehydro-Isolongifolene	C ₁₅ H ₂₂	202	2.74	0.20	1.77
29	25.53	(E)-alpha-Ionone	C ₁₃ H ₂₀ O	192	0.66	0.44	

Table 1. Contd.

30	25.84	beta-Caryophyllene	C ₁₅ H ₂₄	204		1.72		
31	26.13	(-)-alpha-Santalene	C ₁₅ H ₂₄	204	1.71	0.71	3.00	
32	26.98	alpha -Guaiene	C ₁₅ H ₂₄	204	0.58	0.29		
33	27.51	alpha-Caryophyllene	C ₁₅ H ₂₄	204		0.68	1.00	
34	28.67	beta-Cadinene	C ₁₅ H ₂₄	204	0.55	0.35		
35	28.81	(-)-alpha-Gurjunene	C ₁₅ H ₂₄	204		0.31		
36	28.96	beta--Cubebene	C ₁₅ H ₂₄	204	0.41	0.42		
37	29.23	beta-Selinene	C ₁₅ H ₂₄	204	1.15	2.51	3.24	
38	29.47	(+)-Epi-bicyclosesquiphellandrene	C ₁₅ H ₂₄	204	2.11	0.55	2.14	
39	29.68	gamma-Muurolene	C ₁₅ H ₂₄	204		0.66		
40	29.86	(+)-gamma-Gurjunene	C ₁₅ H ₂₄	204	1.21	0.73		
41	30.59	alpha-Muurolene	C ₁₅ H ₂₄	204			2.24	
42	30.87	gamma-Cadinene	C ₁₅ H ₂₄	204	1.18	1.46		
43	31.56	(+)-delat-Cadinene	C ₁₅ H ₂₄	204	2.74	3.72	3.50	
44	32.20	alpha-Calacorene	C ₁₅ H ₂₀	200	0.80	0.88		
45	32.69	Elemol	C ₁₅ H ₂₆ O	222	4.85	3.29	2.71	
46	33.44	gamma -Elemene	C ₁₅ H ₂₄	204			1.88	
47	34.09	(Z)-Nerolidol	C ₁₅ H ₂₆ O	222	0.55	0.67		
48	34.30	(-)-Spathulenol	C ₁₅ H ₂₄ O	220	15.53	10.81	3.55	
49	34.58	Caryophyllene oxide	C ₁₅ H ₂₄ O	220	10.51	11.98	11.26	
50	34.74	(+)-Spathulenol	C ₁₅ H ₂₄ O	220	1.23			
51	35.69	Cedrenol	C ₁₅ H ₂₄ O	220	0.51			
52	36.24	Isoaromadendrene epoxide	C ₁₅ H ₂₄ O	220	1.88	1.85	1.59	
53	37.78	gamma-Himachalene	C ₁₅ H ₂₄	204			1.17	
54	38.00	alpha -Cadinol	C ₁₅ H ₂₆ O	222	1.16	0.61		
55	38.42	trans-Longipinocarveol	C ₁₅ H ₂₄ O	220	0.76	0.51		
56	38.77	tau-Cadinol	C ₁₅ H ₂₆ O	222	2.61	1.49	1.06	
57	39.11	beta-Eudesmol	C ₁₅ H ₂₆ O	222	1.54	1.12		
58	39.33	Juniper camphor	C ₁₅ H ₂₆ O	222	0.75	0.46	3.28	
59	39.53	(+)-Ledene	C ₁₅ H ₂₄	204		1.79		
60	39.63	tau-Muurolol	C ₁₅ H ₂₆ O	222	3.32			
61	40.47	Aromadendrene oxide-(1)	C ₁₅ H ₂₄ O	220		0.67		
62	40.77	Ledene oxide-(II)	C ₁₅ H ₂₄ O	220	1.70	1.63	1.59	
63	53.11	6,10,14-trimethyl-2-Pentadecanone	C ₁₈ H ₃₆ O	268	2.36	1.66	3.13	
64	54.28	Farnesylacetone	C ₁₈ H ₃₀ O	262	0.29	0.22		
65	54.62	Hexadecanoic acid-methyl ester	C ₁₇ H ₃₄ O ₂	270	0.19		0.40	
66	55.11	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256	1.09	1.14	1.38	
67	57.43	Phytol	C ₂₀ H ₄₀ O	296	3.78	2.40	3.75	

Blank: the components were not discovered.

Types of the chemical components of essential oil

The obvious differences of chemical component types of the essential oils were observed between the tested wild plants as well as between the wild and the control "Suxinmei" plants (Table 2). The component numbers of monoterpenes and sesquiterpenes in the wild plant W2 were for 21 and 30, respectively, more than those in W1 for 10 and 27, respectively. Of which, the oxygenated monoterpenes for 14 components and the sesquiterpenes hydrocarbons for 18 types in the former

were more than those in the latter for 10 and 13 ones, respectively. Especially, the monoterpene hydrocarbons were only observed in the W2 plant. However, all the types in the wild plants W1 and W2 were more than those in the variety "Suxinmei" like oxygenated monoterpenes, sesquiterpene hydrocarbons, oxygenated sesquiterpenes and aliphatic only with 4, 9, 7 and 4, respectively. Moreover, the aromatic components existing in the wild plants were absent in "Suxinmei".

The number and relative content of oxygenated diterpenoids were comparatively stable in the wild and

Table 2. Types of chemical components of the leaf essential oil from wild and domestication *C. praecox*.

Grouped types of chemical components	Compound number			Relative content (%)		
	W1	W2	C1	W1	W2	C1
Monoterpene hydrocarbons	-	7	-	-	5.62	-
Oxygenated monoterpenes	10	14	4	26.59	28.47	45.16
Total	10	21	4	26.59	34.09	45.16
Sesquiterpene hydrocarbons	13	18	9	16.72	21.87	19.94
Oxygenated sesquiterpenes	14	12	7	46.90	35.10	25.04
Total	27	30	16	63.62	56.97	44.98
Oxygenated diterpenoids	1	1	1	3.78	2.40	3.75
Aromatic components	2	2	-	0.73	2	-
Aliphatic components	6	5	4	5.28	3.93	6.11
Sum identified	46	59	25	100	99.38	100

-: the components were not discovered.

the variety "Suxinmei" materials because they had the same components and close contents (Table 2). Additionally, the leaf essential oils of both the wild and "Suxinmei" plants were mainly constituted by sesquiterpenes and monoterpenes with both the more numbers and higher relative contents of components. And, their essential oils had higher oxygenated sesquiterpenes and monoterpenes than sesquiterpenes and monoterpene hydrocarbons like 46.90% > 16.72%, 35.10% > 21.87%, 25.04% > 19.94% and 28.47% > 5.62%, even monoterpene hydrocarbons could not be discovered in the W1 and C1 plants (Table 2).

DISCUSSION

In this paper, the yields and chemical components of essential oil in wild *C. praecox* leaves were firstly investigated and compared with those of the domestication variety "Suxinmei". The wild and "Suxinmei" plants of *C. praecox* shared the twenty-two components like: borneol, bornyl acetate, etc, besides possessing the same component number and close relative content of oxygenated diterpenoids. Moreover, they were mainly constituted by the three main components like bornyl acetate, caryophyllene oxide and (-)-spathulenol, as well as characterized by the sesquiterpenes and monoterpenes combined with the high contents of oxygenated sesquiterpenes and monoterpenes. It might be associated with the close relationships between wild and "Suxinmei" plants, because they are all derived from the species *C. praecox*.

And, it was implied that these components of essential oil in the leaves of *C. praecox* might be comparatively stably inherited and expressed in the evolution and domestication processes from wild to cultivar.

Similar to the previously reported results in *Artemisia annua* (Yu et al., 2009; Zhang et al., 2009), *Senecio scandens* (He et al., 2010) and *Sarcandra glabra* (Wu et al., 2009), obvious differences of the essential oils between the wild and domestication *C. praecox* leaves were also observed in the present study. The wild plants possessed higher essential oil content and more abundant chemical components than the variety "Suxinmei" (Figure 1 and Tables 1 and 2). Most chemical components like linalool, (1s)-(-)-camphor, (s)-cis-verbenol, α -terpineol, etc of the essential oils in the wild plants, were absent in "Suxinmei". Not only the oxygenated monoterpenes and sesquiterpenes hydrocarbons in the wild plants were more than those in "Suxinmei", also the monoterpene hydrocarbons and aromatic compounds only existed in the wild materials.

Majority of these components belonged to terpenoids with good flavor, and therefore might constitute the special fragrance of essential oil. These implied that the excellent genetic factors encoding high contents and rich chemical components of essential oil in the leaves might been lost or unexpressed in "Suxinmei" because this variety with pure yellow flowers was the natural mutant from wild *C. praecox* with speckles on the inside petals. It is needed to be further studied whether or not the components existing in wild plants but absent in the control "Suxinmei", are associated with the red or purple speckles of flowers. In addition, both the yield, and component numbers and types of essential oil from the wild W1 were different from those from the W2 (Figure 1 and Tables 1 and 2). It revealed, in a way, the genetic diversity in wild plants of *C. praecox*.

Aromas of essential oil are prominently defined by some fragrance molecules (Li, 2009a). The present research showed that, the chemical components of essential oil in the leaves of *C. praecox* were obviously

different from those of other reported species like *C. nitens* (Zhan and Xu, 2006; Xu et al., 2006; Cao et al., 2008; Shu et al., 2010), and *C. zhejiangensis* (Ou and Mai, 2010) in *Chimonanthus*, as well as those in other reported organs like flowers (Jing et al., 2005; Farsam et al., 2007; Li et al., 2008; Si et al., 2010; Zhao et al., 2010) and seeds (Li and Zhang, 2006) of *C. praecox*. Of which, many had enjoyable aroma. For example, bornyl acetate, as one of main components, has the fragrance similar to pine. Phytol has herbaceous aroma, and eucalyptol has a strong wood, coriander-like scent. And, n-hexadecanoic acid has a very sweet smell as wax esters. Likewise, caryophyllene flavor is much closer to the original clove odour and wood-aromatic flavour, (z)-nerolidol flavor is a faint, sweet and lasting floral odor similar to those of rose and apple. Moreover, damascenone with a honey, spice, lilac and rose-like fragrance, is an important fragrant chemical used in perfumery (Li, 2009b; Ni et al., 2008).

On the other hand, many chemical components of the leaf essential oils from *C. praecox* have a significant antitussive, expectorant and antiasthmatic effects, such as bornyl acetate, (-)-spathulenol, terpinen-4-ol, eucalyptol, pinene (Ou et al., 2003). And, elemene have obvious anti-tumor effect (Gu, 1994). In addition, terpinen-4-ol, caryophyllene, eucalyptol and pinene have anti-inflammatory, antibacterial and analgesic effects (Ou et al., 2003). Apparently, same as some published results, the essential oil in the leaves of *C. praecox* also has important developmental foreground in cosmetic, medicine, food flavor or fragrance industries, especially the wild *C. praecox* because of containing richer chemical components of enjoyable flavor and curative effect, than the variety "Suxinmei". Therefore, it is of importance to powerfully protect, rationally develop, and sustainably utilize the wild germplasm resources of *C. praecox*.

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