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Full Length Research Paper

Monoterpene composition of flower and bract from *Houttuynia cordata*

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Houttuynia cordata **Thunb. is an aromatic and medicinal wild herb. However, little information is available about the monoterpene composition of the flowers and bracts. Gas chromatography-mass spectroscopy (GC-MS) analyses led to the identification of 15 monoterpenes in the essential oils of *H. cordata* accessions w01-39 [chemotype decanal (D)], w01-94 [chemotype myrcene (M)] and w01-100 (chemotype M). 13 to 14 monoterpenes were detected in the flowers of the three *H. cordata* accessions, respectively. 10 and 11 monoterpenes were found in the bracts of w01-94 and w01-100, respectively, while only 7 monoterpenes were observed in the bracts of w01-39. The contents of total monoterpenes in the flowers and bracts of chemotype D and M were all high (33.60 to 56.15%), while it only showed little variations (52.39 to 56.15%) in the flowers either within chemotype M or between chemotype D and M. Interestingly, although the leaves of w01-39 produced very low levels of monoterpenes, the flowers and bracts were rich in monoterpenes. Like leaves, both the flowers and bracts were dominated by monoterpene hydrocarbons represented mainly by β-myrcene and β-phellandrene. It therefore concluded that both the flowers and bracts could prove to be a useful source of monoterpenes.**

Key words: Bracts, flowers, essential oil, *Houttuynia cordata*, monoterpenes.

INTRODUCTION

Houttuynia cordata Thunb. is an aromatic and medicinal herb with great importance in the pharmaceutical and fragrance industries. It is also a popular wild vegetable in Asian countries such as China (Wu et al., 2005), Korea (Kim et al., 2001), India (Chakraborti et al., 2006), Vietnam (Ogle et al., 2003) and Thailand (Nuengchamnong et al., 2009). In China it has been used as a traditional Chinese medicine (TCM) for hundreds of years and is effective in curing complex diseases such as pneumonia, hemoptysis, severe acute respiratory syndrome (SARS) and influenza virus (Hayashi et al., 1995; Lau et al., 2008; Lu et al., 2006). It therefore has been identified as one of the most potential medical and edible plant resources by the Chinese State Health Department.

It is well known that the leaf and stem of *H. cordata* produce essential oil and has been mainly used as TCM. Monoterpenes are one of the active constituents in the essential oil of *H. cordata* (Crowell, 1997; Stark et al., 1995).

While our previous study reported that the species from different populations exhibited highly complex variation in chromosome number (Wu et al., 2003) and in essential oil compositions (Chen et al., 2008), and monoterpene components in the essential oil of *H. cordata* populations with different chromosome numbers are distinguished (Chen et al., 2008).

Based on the monoterpene compositions in the leaves, *H. cordata* populations from Sichuan and Chongqing province in China have been divided into two chemotypes. Chemotype myrcene (M) processes essential oil rich in monoterpenes and inversely chemotype decanal (D) has a lower content of monoterpenes. Interestingly, all populations with chromosome number less than 80 are classified into chemotype M and the populations grouped into chemotype D have chromosome number less than 80. Moreover, different populations with the same chromosome numbers may show variation in the monoterpene compositions in their leaf and stem (Chen et al., 2008). However, little information is available about

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Table 1. The accessions numbers, localities, chromosome numbers and chemotypes of *H. cordata* used in the present study.

No.	Localities	Chromosome numbers	Chemotypes[a]
w01-39	Hanlin, Pengshui, Chongqing	54	D
w01-94	Wangyu, Ya'an, Sichuan	90	M
w01-100	Qionglai, Sichuan	90	M

[a] D and M represent decanal and myrcene chemotypes, respectively.

he monoterpene compositions of the flower and bract from *H. cordata*. These hamper the application of their flower and bract in the medicinal and fragrance industries. This study therefore attempts (1) to investigate the monoterpene constituents of their flowers and bracts, and (2) to characterize the variations in the contents of monoterpenes of flower and bract from different chemotypes of *H. cordata*, and (3) from different populations with the same chromosome numbers and chemotypes.

MATERIALS AND METHODS

Plant materials and growth conditions

In 2009, 3 different accessions of *H. cordata* from China were used in this study. Their accessions numbers, localities, chromosome numbers and chemotypes are presented in Table 1. All of the plants were planted in earthen pots (75 cm diameter) containing a mixture of soil, sand and perlite (2: 2: 1) and the pots were kept in the field of Sichuan Agricultural University at Ya'an (latitude 29° 59′ 08″N, longitude102° 58′ 56″E, and altitude 595 m), Sichuan province, China. Water was uniformly added to each pot until field moisture capacity for the uniformity in growth. The flower, bract and third fully expanded leaves were collected randomly from uniform plants during the actual florescence in April.

Essential oil extraction

Samples (50 g FW) were subjected to hydrodistillation for 4 h to extract essential oils by using a Clevenger apparatus (Clevenger, 1928). 2 ml ethyl acetate was used as solvent. The ethyl acetate layers were collected to the constant volume of 10 ml using ethyl acetate. The extracts were dehydrated by passing through anhydrous sodium sulphate and sealed with parafilm at 4°C in dark until used for gas chromatography-mass spectrometry (GC-MS) analysis.

GC-MS analysis

GC-MS analysis was performed on a GC (Agilent, 6890N) interfaced with a mass selective detector (Agilent, 5973B) equipped with an Agilent HP-5MS silica capillary column (30 m × 0.25 mm and film thickness 0.25 µm). The carrier gas was helium with a constant flow rate of 1 ml min^{-1}. Injector temperatures were 250°C. A split injection with a ratio of 1:50 was used. The oven temperature was set at 70°C for 2 min, programmed to 110°C at a rate of 10°C min^{-1}, and held at 110°C for 4, then heated to 220°C at a rate of 10°C min^{-1}, isothermal at this temperature for 4 min. The mass spectra operating parameters were as follows: ionisation potential, 70 eV; interface temperature, 280°C; acquisition mass range, 50 to 450. Relative percentage amounts of monoterpene components in the volatile oil were evaluated from the total peak area by apparatus software. Identification of components was based on the comparison of their mass spectra and retention time with those of authentic compounds and by computer matching with NIST 2.0 and Wiley libraries as well as by comparison of the mass spectral data with those reported in the authentic references (Chen et al., 2008).

RESULTS AND DISCUSSION

GC-MS analyses led to the identification of 15 monoterpenes in the essential oils of three *H. cordata* accessions (Table 2). The monoterpenes of leaves were additionally determined as control, and 6, 15 and 15 monoterpenes were found in the leaves of w01-39, w01-94 and w01-100, respectively. The data are in accord with the chemotype principles of *H. cordata* (Chen et al., 2008). In chemotype D, w01-39, the number of monoterpenes of the bracts is nearly as low as that of the leaves, while the flowers produced as many as 13 monoterpenes. In chemotype M, the analyses of the essential oils of w01-94 showed the presence of 13 and 10 monoterpenes in the flowers and bracts, and the number were close to that of the leaves. The parallel changes were observed in the flowers and bracts of the other accession of chemotype M, w01-100. In general, the monoterpene constituents of the flowers of all *H. cordata* accessions were abundant, while significant changes of the number of monoterpenes were observed in the bracts between chemotype D and M.

The content of total monoterpenes in the flowers of three *H. cordata* accessions was generally higher than that in the bracts, respectively. Although the variations of the total monoterpene content in the leaves were significant among the three *H. cordata* accessions, little variations in the content of total monoterpenes of the flowers were observed either within chemotype M or between chemotype D and M. While significant changes of total monoterpene contents in the bracts were observed between chemotype D and M, and even within chemotype M. Interestingly, although the leaves of w01-39 produced very low levels of monoterpenes, the total monoterpene contents of both the flowers and bracts were high, 56.15 and 33.6%, respectively. It is likely that these chemical variations are due to difference in expression of monoterpene synthase genes (Fischbach et al., 2002; Guitton et al., 2010).

Table 2. Monoterpene profile of flowers, bracts and leaves of three *H. cordata* accessions.

No.	Monoterpenes	W01-39			W01-94			W01-100		
		Flower	Bract	Leaf	Flower	Bract	Leaf	Flower	Bract	Leaf
1	α-Pinene	4.44	1.07	0.25	4.48	1.23	1.32	3.50	2.05	2.76
2	Camphene	0.37	—	—	1.12	—	0.24	0.61	—	0.29
3	β-Phellandrene	10.01	4.71	0.50	8.83	4.26	5.02	12.90	8.37	16.87
4	β-Pinene	7.02	1.63	0.36	4.47	1.17	1.19	3.56	2.17	2.92
5	β-Myrcene	24.07	19.60	1.43	25.79	26.57	13.54	21.28	20.64	16.03
6	α-Phellandrene	t	—	—	—	—	t	t	—	0.13
7	α-Terpinene	1.47	1.05	—	0.71	1.06	1.16	1.51	2.28	2.64
8	p-Cymene	—	—	—	—	—	0.25	—	0.29	0.41
9	D-Limonene	1.29	—	—	1.51	0.26	0.25	0.95	—	t
10	β-Ocimene	—	—	—	1.66	2.62	3.03	2.62	5.12	8.62
11	γ-Terpinene	2.37	1.85	0.19	1.20	1.89	2.07	2.76	3.88	4.30
12	4-Terpinenyl acetate	0.20	—	—	0.18	—	t	0.29	—	0.13
13	Terpinolene	0.61	—	—	0.43	0.44	0.52	0.67	0.86	1.04
14	4-Terpineol	3.98	3.69	0.35	1.88	3.64	3.89	4.58	7.25	7.58
15	α-Terpineol	0.33	—	—	0.14	—	0.23	0.26	0.48	0.43
	Hydrocarbons	51.65	29.91	2.73	50.18	39.50	28.58	50.35	45.66	56.03
	Alcohols	4.30	3.69	0.35	2.03	3.64	4.12	4.84	7.73	8.01
	Esters	0.20	—	—	0.18	—	—	0.29	—	0.13
	Total	56.15	33.60	3.09	52.39	43.14	32.70	55.49	53.39	64.16

t = trace (<0.1%),(–) = not detected. All values are the average of three repeats.

As the same as leaves, the flowers and bracts of the three *H. cordata* accessions were all dominated by monoterpene hydrocarbons represented mainly by β-myrcene and β-phellandrene, and only had a small share of monoterpene alcohols and esters. Previous studies have indicated the presence of monoterpenes is a significant quantity for *H. cordata* because of the aromatic properties and pharmacology activities (Chen et al., 2008; De Feo et al., 2003). From the above results, it therefore is concluded that both the flowers and bracts of chemotype D and M could prove to be a useful source of monoterpenes.

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