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ORIGINAL ARTICLE

A Special Aromatic *Chrysanthemum* Breed with High Content of Thujone

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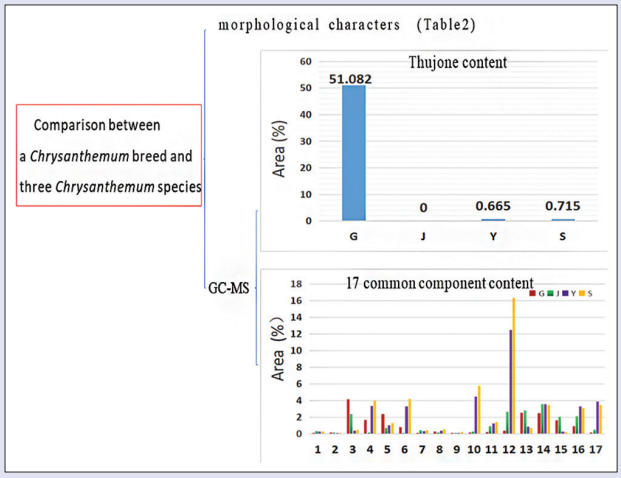
ABSTRACT

Background: A cultivar of *Chrysanthemum*, whose whole plant has strong and special fragrance, was found in Jiugong Mountain area, Xianning City, Hubei Province. **Objective:** The objective of the study was to determine taxonomic status of the cultivar in genus *Dendranthema*. **Materials and Methods:** The differences of morphological characteristics and volatile oil components between this cultivar and *Dendranthema indicum, D. indicum* var. *aromaticum*, and *Dendranthema molifolium* cv. "Jinju" were compared. **Results:** The cultivar was found to be a *Chrysanthemum* breed with unisexual flowers. The volatile oil content was 0.25% in its stem and leave of the breed and about 1.81% in its inflorescence which was much higher than that of other 3 species. There are 17 common components among volatile oils in inflorescence of the four *Chrysanthemum* species, but the thujone content of this cultivar is much higher than other three species. **Conclusions:** The cultivar is a new, cultivated *Chrysanthemum* breed, rich in volatile oil and thujone content, and named as *Dendranthema morifolium* (Ramat.) Tzvel. cv. "Jiugong Xiangju."

Key words: Cultivated breed, *Dendranthema molifolium,* tujone, volatile oil

SUMMARY

- A cultivar of *Chrysanthemum* which was found in Jiugong Mountain area, Xianning City, Hubei Province can be identified by comparing morphological characteristics and volatile oil components with *Dendranthema indicum, D. indicum* var. *aromaticum*, and *Dendranthema molifolium* cv."Jinju". The cultivar is a new cultivated *Chrysanthemum* breed, rich in volatile oil and thujone content,and named as Dendranthema morifolium (Ramat.) Tzvel. cv. "Jiugong Xiangju".



Abbreviations used: G: *Dendranthema morifolium* (Ramat.) Tzvel. cv. "Jiugong Xiangju"; J: *Dendranthema molifolium* (Ramat.) Tzvel. cv. "Jinju"; Y: *Dendranthema indicum* (L.) Des Moul; S: *Dendranthema indicum* var. *aromaticum* Q. H. Liu et S. F. Zhang.

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INTRODUCTION

Medicinal *Chrysanthemum* is the capitulum of *Dendranthema molifolium* (Ramat.) Tzvel. (Family Compositae) and it is a famous medicinal and edible plant material with a long history of cultivation in China. In Xianning City of Hubei Province in China, a *Chrysanthemum* cultivar was found, which stems, leaves, and flowers have strong fragrance. The cultivar is cultivated in the geographical and ecological area around the Jiugong Mountains which is located in Hubei and Jiangxi province and has long application history as both edible and medicinal *Chrysanthemum* in Xianning City of Hubei Province and Jiujiang City of Jiangxi Province. It is believed that the cultivar has a good effect on relieving eye fatigue and improving eye diseases. Due to its aromatic smell of stems and leaves and growing in the Jiugong Mountains, it is named as Jiugong Xiangju (JGXJ).

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In *Dendranthema* genus, plants with fragrance throughout the whole plant are relatively rare; two species of them are found in Hubei Province. They are *Dendranthema indicum* var. *aromaticum* Liu *et al.*[1] and *D. molifolium* (Ramat.) Tzvel. cv. "Jinju."[2] However, according to our survey in Jiangling county, Jingzhou city, the stems and leaves of the gold *Chrysanthemum* have no aroma. Therefore, in this article, morphological characteristics of these species and contents of the volatile oils in their inflorescences were compared, and the compounds of volatile oil in the stems, leaves, and inflorescences of the cultivar, JGXJ, were analyzed to determine its location in *Dendranthema* genus in plant taxonomy.

MATERIALS AND METHODS

Materials

Ten samples of inflorescences, come from JGXJ, *D. indicum*, *D. indicum* var. *aromaticum*, and *D. molifolium* cv. "Jinju," were separately collected from Jiujiang City, Jiangxi Province, and Xianning City, Tongshan or Badong country, Hubei Province and identified by Professor Keli Chen of the Hubei University of Chinese Medicine [Table 1]. Samples G5 and G6 come from the stems and leaves of JGXJ, collected from as G1 and G3, respectively.

Reagents and instruments

Reagents used were anhydrous ether (analytically pure, Tianjin Deen Chemical Reagent Co., Ltd.) and anhydrous sodium sulfate (analytically pure, Sinopharm Chemical Reagent Co., Ltd.).

Instruments used were pulverizer, high-frequency desktop LCD ultrasonic cleaner; MS204S one ten-thousandth analytical balance (METTLER TOLEDO); electronic thermostat electric heating jacket (Tianjin Taisite Instrument Co., Ltd.); and Thermo Scientific™ TRACE™ 1310 ISQ™ LT Single Quadrupole GC-MS (Thermo Fisher, USA), Thermo TG-1701MS capillary column (0.25 μm × 0.25 mm mm30 m).

Preparation of plant extracts

Each sample powder (50 g) was soaked with distilled water (600 mL) for 1.5 h, sonicated for 40 min, and then placed into a 2 L round-bottomed flask and extracted by steam distillation for 8 h, and then, the distillate was collected and extracted with ether. After the ether layer was dried with anhydrous sodium sulfate, the ether was evaporated to obtain pure volatile oil. It was placed in a sealed glass bottle and stored at 4°C until analysis.

Sample solution

Before injection, 100 μl of volatile oil of each sample was dissolved in 1 ml of chromatographically pure ether, filtered through a 0.45 μm microporous membrane, and for use.

Gas chromatography–mass spectrometry analysis conditions

The GC column was Thermo TG-1701MS capillary column (0.25 μm × 0.25 mm 30 m), injection volume was 1.0 μL, split ratio was 50:1, carrier gas was high purity helium, flow rate was 1.0 mL·min^{-1}, and inlet temperature was 250°C. Programmed temperature included initial temperature at 50°C held for 5 min and heated to 250°C at 2°C ·min^{-1} for 5 min.

Mass spectrometry (MS) transmission line temperature was 250°C, ionization mode was electron ionization source, electron energy: 70 eV, ion source temperature: 200°C, ion scanning range: 50–500 (*m/z*), and solvent delay time: 3 min.

Qualitative and quantitative analysis

The mass spectral data of the obtained chromatographic peaks were qualitatively searched by component analysis Xcalibur software and Mainlib database, and the relative percentage of each component in the volatile oil was calculated according to the peak area normalization method.

RESULTS

Morphological characteristics

According to our observation, the morphological characteristics of JGXJ are as follows: the plant height is 40–50 cm. Stem is erect, sparsely pilose, and densely in the inflorescence branches. Leaf blade is ovate to broadly ovate, fissure to mid-crack, lobes apex slightly acute, base slightly cuneate (not truncate), and both surfaces dark green and sparsely pubescent. Involucral bracts are two-layered, the outer layer is ovate or ovate-triangular, outside tomentose, and bracts margin brownish broadly membranous. Capitula is 2.5–4 cm in diameter. In the outermost layer, there are ray florets, female, white, and 11–12 mm wide. The others are tubular florets, all female, in the outer florets yellow-white or white, yellow in the middle, all white when fully matured, and apex 5 denticulate. The flowering period is from November to December.

The morphological characteristics of four species, including JGXJ, *D. indicum* var. *aromaticum*, *D. indicum*, and *D. molifolium* cv. "Jinju" were compared, as shown in Table 2.

Volatile oil yield

The volatile oils of 12 *Chrysanthemum* samples were extracted. The volatile oil in stem and leaf of JGXJ is yellowish brown with a yield of 0.25%. The yield and color of volatile oil of 10 inflorescences samples are shown in Table 1. (The volatile oil yield [%] = constant weight of volatile oil [g]/sample mass [g].)

Table 1: Similarity analysis of 10 samples compared with common pattern

Number	Sample	Collecting place	Yield (%)	Color	Similarity
G1	JGXJ	Jiujiang Jiangxi	1.75	Bright yellow	0.986
G2	JGXJ		1.79	Bright yellow	0.925
G3	JGXJ	Tongshan Hubei	1.80		0.990
G4	JGXJ		1.89		0.974
J1	*D.* morifolium cv. 'Jinju'	Tongshan Hubei	0.54	Yellow	0.110
J2	*D.* morifolium cv. 'Jinju'		0.73		0.110
S1	*D. indicum* var. *aromaticum*	Badong Hubei	1.63	Reseda	0.082
S2	*D. indicum* var. *aromaticum*		1.51		0.080
Y1	*D. indicum*	Tongshan Hubei	1.01	Bottle green	0.091
Y2	*D. indicum*		0.91		0.082

D. indicum: *Dendranthema indicum*; *D.* morifolium: Dendranthema *morifolium*; JGXJ:Jiugong Xiangju

Table 1 shows that there were some differences in the volatile oil content and color of different samples. The yield of volatile oil of JGXJ inflorescences was significantly higher than that of the other three *Chrysanthemum*s species and also higher than that of its stems and leaves.

Comparative analysis of compounds

GC-MS analysis of the volatile oils 10 inflorescences samples showed that the volatile oils contained 17 same compounds in the inflorescences of all the four species of *chrysanthemum*. However, it is noticed that, among them, the volatile oil of *D. molifolium* cv. "Jinju" does not contain thujone [Table 3].

Similarity analysis of gas chromatography spectra

Four batches of GC data from the volatile oil of JGXJ (G1–G4) [Figure 1] were introduced into the software of Chinese Medicine Chromatographic Fingerprint Similarity Evaluation System (2004 A Edition) to generate a common pattern map of JGXJ [Figure 2]. Then, the similarity analysis was carried out on the GC maps of the other six samples of other three *Chrysanthemum* species and the common pattern map® [Figure 3]; the results are shown in Table 1.

Gas chromatography–mass spectrometry analysis of volatile oil from Jiugong Xiangju

The volatile oils of six JGXJ samples (G1–G6) were analyzed by GC-MS to obtain the total ion chromatogram, and then, qualitative and quantitative analysis was carried out according to the above method, as shown in Table 4.

As shown in Table 4, 27 chemical constituents were identified in the volatile oils of stems, leaves, and inflorescences of JGXJ, and terpene compounds were the main components. The oil content and composition of stems and leaves were different from that of the inflorescences, and three compounds are found only in leaves and stems and can be used to differentiate the volatile oil of inflorescences from that of leaves and stems. Moreover, there are 24 same components which are contained

Table 2: The morphological characteristics of four *Chrysanthemum* species

	JGXJ	*D. indicum* var. *aromaticum*	*D. indicum*	D. morifolium cv. 'Jinju'
Stem height (cm)	40-50	20-40	25-100	30-90
Leaf				
Color	Dark green	Green	Light green	Dark green
Shape	Base slightly cuneate (not truncate)	Base somewhat cordate	Base truncate, somewhat cordate or broadly cuneate	Base slightly cuneate (not truncate)
Surface	Both sides of leaves sparsely pubescent	The upper leaf veins raised, and the lower glands depressed. others as the same as *D. indicum*	Both sides of leaves sparsely pubescent, slightly more below	Both sides of leaves sparsely pubescent
Capitula				
Diameter (cm)	2.5-4	1-1.2	1.5-2.5	2-3
Ray florets (mm)	11-12; white	4-6; yellow	10-13; yellow	10-13; white
Tubular florets	Yellow to white, white when fully matured; unisexual	Yellow; bisexual		Yellow or yellow-white; bisexual

D. indicum: *Dendranthema indicum*; *D.* morifolium: Dendranthema *morifolium*; JGXJ:Jiugong Xiangju

Table 3: Chemical constituents and relative contents of volatile oils in 10 samples

Number	RT (min)	Compound name	Area percentage									
			G1	G2	G3	G4	J1	J2	S1	S2	Y1	Y2
1	8.20	α-Pinene	0.03	0.02	0.01	0.04	0.02	0.64	0.33	0.22	0.22	0.33
2	9.20	Camphene	0.10	0.06	0.03	0.11	-	0.16	0.09	0.11	0.11	0.17
3	11.04	Sabenene	0.58	0.89	0.98	1.56	-	-	0.06	0.07	0.07	0.09
4	12.25	β-Pinene	0.35	0.14	0.26	0.39	-	-	0.08	0.08	0.08	0.10
5	15.28	Eucalyptol	5.40	2.60	3.89	4.75	0.02	4.79	0.40	0.40	0.49	0.46
6	24.31	Thujone	60.94	52.15	45.32	45.92	-	-	0.67	0.66	0.67	0.76
7	26.85	Camphor	1.09	1.72	1.93	1.98	0.01	0.38	3.25	3.49	3.76	4.27
8	27.88	Terpinen-4-ol	1.79	2.75	2.42	2.51	0.15	1.25	1.00	1.04	1.52	1.15
9	29.08	Borneol	0.66	0.79	0.89	0.98	0.03	0.25	2.78	3.86	4.28	4.22
10	29.39	p-Mentha-1,5-dien-8-ol	0.06	0.02	0.02	0.08	0.11	0.74	0.25	0.46	0.51	0.36
11	30.20	alpha-Terpineol	0.22	0.23	0.27	0.4	0.07	0.27	0.22	0.55	0.61	0.54
12	31.39	Myrtenal	0.06	0.07	0.09	0.03	0.02	0.26	0.11	0.12	0.22	0.24
13	31.76	Myrtenol	0.09	0.17	0.17	0.19	-	0.28	4.00	4.96	5.62	5.99
14	34.20	Bornyl acetate	0.16	0.22	0.26	0.21	0.23	1.67	1.11	1.39	1.55	1.29
15	42.39	(E)-β-Famesene	0.23	0.23	0.54	0.47	4.18	1.15	11.98	12.96	12.63	20.0
16	45.68	(Z, E)-a-Farnesene	0.04	0.03	0.12	0.11	-	-	0.90	1.15	1.17	0.85
17	45.86	Naphthalene	1.68	3.19	3.54	1.88	2.90	2.76	0.90	0.81	0.75	0.67
18	46.69	β-sesquiphellandrene	2.39	1.13	3.19	3.27	5.78	1.38	3.52	3.62	3.53	3.43
19	56.62	.tau.-Muurolol	1.11	1.98	1.78	1.7	2.39	1.79	0.27	0.29	0.32	0.19
20	73.35	(Z)-2-(Hexa-2,4-diyn-1-ylidene)-1,6-dioxaspiro[4.4]non-3-ene	1.12	0.39	0.21	2.05	3.17	1.09	3.20	3.39	3.02	3.14
21	75.32	(E)-2-(Hepta-2,4-diyn-1-ylidene)-1,6-dioxaspiro[4.4]non-3-ene	0.08	0.09	0.4	0.12	0.84	0.21	3.88	3.97	3.4	3.49

RT: Retention time

in stems, leaves, and inflorescences, and among them, thujone was the main component in the volatile oil of leaves and stems (>30%) and inflorescences (>45%).

DISCUSSION AND CONCLUSION

According to the results of the analysis from Table 3, the content of thujone in the volatile oil of inflorescences of JGXJ is as high as 45% or more. It is the highest content of thujone found in plants of genus *Dendranthema* so far, so JGXJ can be used as a new source plant for extracting thujone. Thujone, a kind of monoterpene ketones, has been proved to promote the proliferation of spleen cells, thymocytes, and production of cytokines (such as interleukin-2 and interferon-gamma),[3] and to have anti-tumor ability.[4,5]

According to the reports, thujone has been found in volatile oils of *D. indicum*[6-9] and its variety *D. indicum* var. *aromaticum*[10-12] but not found in that of species series of *D. molifolium*. Hence, it is supposed that JGXJ has a closer genetic relationship with *D. indicum* than *D. molifolium*. However, the content of thujone in volatile oils of JGXJ inflorescences was higher than 45%, much higher than that from *D. indicum* and *D. indicum* var. *aromaticum*. Moreover, there are 21 same components in

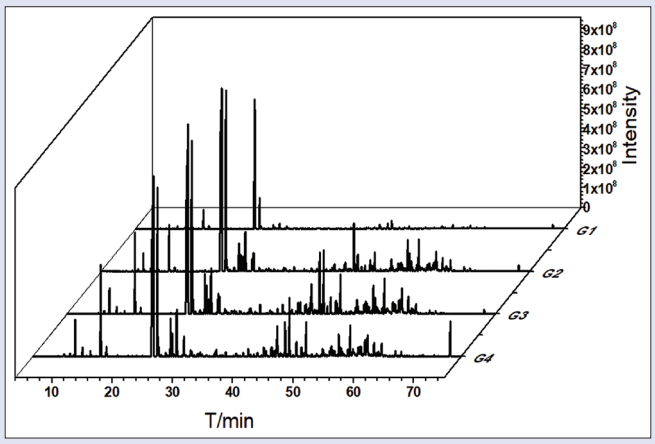

Figure 1: Gas chromatography–mass spectrometry superposition map of volatile oil of Jiugong Xiangju

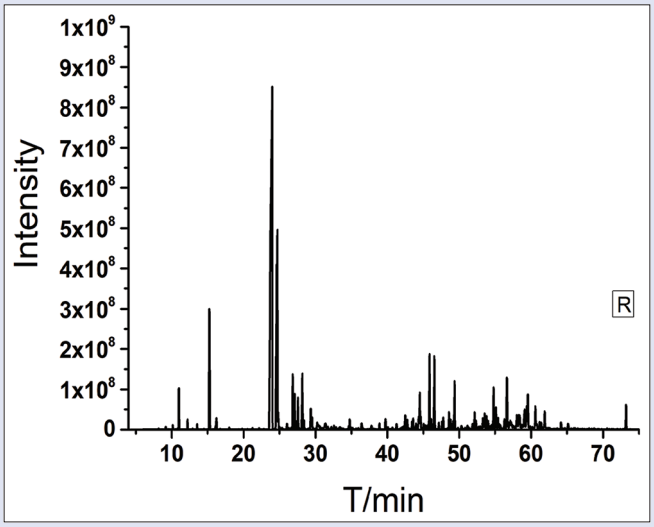

Figure 2: Gas chromatography–mass spectrometry standard map of volatile oil of Jiugong Xiangju

Table 4: Composition and relative content of volatile oil in inflorescence, stem, and leaf of JGXJ

Number	RT (min)	Compound name	Area %					
			G5	G6	G1	G2	G3	G4
1	11.04	Sabenene	0.36	0.04	0.58	0.89	0.98	1.56
2	12.25	β-Pinene	1.24	0.14	0.35	0.14	0.26	0.39
3	15.28	Eucalyptol	2.48	1.33	5.40	2.60	3.89	4.75
4	24.31	Thujone	30.24	35.52	60.94	52.15	45.32	45.92
5	24.81	1,5,7-Octatrien-3-ol, 3,7-dimethyl-	0.18	0.26	0.65	0.48	0.32	0.53
7	26.85	Camphor	0.48	0.92	1.09	1.72	1.93	1.98
8	27.11	cis-Sabinol	0.29	0.59	0.41	1.01	1.37	1.21
9	27.45	1-Isopropyl-4-methylbicyclo[3.1.0]hexan-3-ol	0.08	0.36	0.07	0.84	0.67	0.11
10	44.61	Terpinen-4-ol	0.57	1.23	1.79	2.75	2.42	2.51
11	29.08	Borneol	0.33	0.53	0.66	0.79	0.89	0.98
12	29.23	(-)-Thujol	0.09	0.48	0.09	1.07	0.76	0.07
13	30.20	alpha-Terpineol	0.10	0.16	0.22	0.23	0.27	0.40
14	39.80	Caryophyllene	3.04	2.10	0.24	0.13	0.44	0.39
15	42.39	(E)-β-Famesene	4.8	2.99	0.23	0.23	0.54	0.47
16	44.61	α-Zingiberene	5.51	3.61	0.44	0.09	0.23	0.46
17	44.71	α-Curcumene	1.40	1.67	-	-	-	-
18	45.68	(Z, E)-a-Farnesene	0.69	0.3	0.04	0.03	0.12	0.11
19	55.09	Epicubenol	0.31	0.54	0.31	1.22	0.78	0.49
20	56.30	zingiberenol	0.66	0.63	0.19	0.41	0.31	0.37
21	56.62	.tau.-Muurolol	1.00	1.68	1.11	1.98	1.78	1.7
22	57.54	α-Cadinol	0.20	0.24	-	-	-	-
23	58.75	alpha-Bisabolol	0.55	0.43	0.03	0.10	0.11	0.09
24	61.78	4-(1,5-Dimethylhex-4-enyl) cyclohex-2-enone	0.45	0.55	-	-	-	-
25	64.13	(6R,7R)-Bisabolone	0.14	0.21	0.13	0.24	0.17	0.27
26	73.35	(Z)-2-(Hexa-2,4-diyn-1-ylidene)-1,6-dioxaspiro[4.4]non-3-ene	5.3	4.52	1.12	0.39	0.21	2.05
27	75.32	(E)-2-(Hepta-2,4-diyn-1-ylidene)-1,6-dioxaspiro[4.4]non-3-ene	1.32	0.71	0.08	0.09	0.40	0.12

RT: Retention time

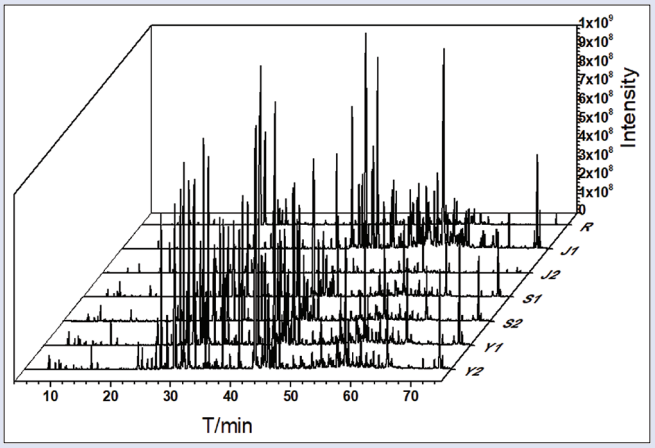

Figure 3: Gas chromatography–mass spectrometry stack map of volatile oil of four Chrysanthemum species

the volatile oils of inflorescences of JGXJ and two species of *D. indicum*, but the similarity of the volatile oil components between JGXJ and the two species were only 0.080%–0.110%. Hence, in chemical composition, the breed of JGXJ is obvious different from *D. indicum* and *D. indicum* var. *aromaticum*.

According to Flora of China,[13] *D. indicum* is a wild species, propagates mainly by seeds, its ray florets with only pistil flowers, but its tubular florets with both pistil and stamen and all yellow, while *D. molifolium* is pluralistic in source and may come from cross-breeding between species and propagates mainly by stem or rhizome. According to the morphological observation for JGXJ, all flowers in its inflorescences are unisexual no matter the ray or tubular florets, and without seeds its propagation depends on asexual reproduction. The average diameter of its inflorescences is 2.5–4 cm, larger than that of *D. indicum*. All ray florets are white and the tubular flowers are white when completely mature, while all the tubular florets of *D. indicum* are yellow. Hence, the characteristics of the reproductive organ flowers of JGXJ are significantly different from those of *D. indicum* in structure and morphology but significantly compatible with those of *D. molifolium*. Therefore, even though JGXJ is only 40–50 cm tall and contains thujone, it can still and only be identified as a cultivated breed of *D. molifolium*, namely here as *Dendranthema morifolium* (Ramat.) Tzvel. "Jiugong Xiangju" cv. nov. Of course, JGXJ maybe is a hybrid of *D. indicum* (or its variants) and a breed of *D. molifolium* for containing thujone and with strong aromas in stems and leaves. To have an insight into the breed of *D. molifolium* and JGXJ, further work is needed in genetic structure and chemical components including flavonoids and organic acids.

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Conflicts of interest

There are no conflicts of interest.

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