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Original Research Article

Quality comparison of traditional Chuanxiong produced in Dujiangyan City and Sichuan ProvinceC and Chuanxiong from other areas, based on analysis of volatile oil, total alkaloids and total ferulic acid contents

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Abstract

Purpose: *To compare the qualities of Chuanxiong from different production areas (authentic and traditional production areas in Dujiangyan, Sichuan Province) and other non-authentic production areas, as well as different germplasm sources, using a combination of methods.*
Methods: *A fingerprint spectrum of volatile oils was established, and the qualities of the chuanxiong samples were compared using gas chromatography (GC), while gas chromatography-mass spectrometry (GC -MS) was used to analyze the chemical compositions of the volatile oils.*
Results: *There were significant differences in the chemical compositions of volatile oils of Chuanxiong from different plants. Most of the qualities of Chuanxiong were higher after transplantation to the germplasm nursery at Dujiangyan, Sichuan. There was increase in the contents of total alkaloids and total ferulic acid in some chuanxiong transplanted to the germplasm nursery.*
Conclusion: *This is the first study that compares the quality of Chuanxiong from different producing areas using a combination of several methods. These methods are feasible and effective, and the results provide a reference for research on standardization and quality control of Chuanxiong.*

Keywords: *Chuanxiong, Volatile oil, Total alkaloids, Ferulic acid, Fingerprint, Dujiangyan City*

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INTRODUCTION

Chuanxiong rhizoma (*chuanxiong*) is derived from the dried rhizome of *Ligusticum chuanxiong* Hort [1], which was first recorded in *Shen Nong's classic of Materia medica*. *Chuanxiong* originated in the Song dynasty and was considered better than others in *ben cao tu jing* which was written by Su song. It was the first time of appearance of "*chuanxiong*" instead of "*xiongqiong*" in the contents of *tang ye ben cao* during Yuan dynasty [2]. Guanxian (now Dujiangyan, Sichuan Province, China) gradually became the main producing area of *chuanxiong* from the Song dynasty, according to the textual research. *Chuanxiong* is a cultivated breed which resources are rich and widespread. It is produced mainly in Dujiangyan, Pengzhou,

Chongzhou, and Xindu, Sichuan Province, China. *Chuanxiong* is also cultivated in Yunnan, Jiangxi and Gansu Provinces, China. *Chuanxiong* from different places differ in quality. The quality of Chinese medicine is important for ensuring the safety and effectiveness of clinical medication. Therefore, there is a clear need for production of high-quality *chuanxiong*. In this study, *chuanxiong* samples were collected from different sources and the chemical compositions of their volatile oils were determined. Then, the contents of volatile oil, total alkaloids, and total ferulic acid in *chuanxiong* from different origins were compared. In addition, comparisons were made between the contents of volatile oil, total alkaloids, and total ferulic acid in *chuanxiong* from their original sources, and the contents of these components after transplantation of the *chuanxiong* resources to the germplasm nursery in Dujiangyan, Sichuan Province.

EXPERIMENTAL

Chemicals and reagents

Reference standards of (+) - camphor, ligustrazine hydrochloride and ferulic acid were purchased from National Institute for Food and Drug Control (Beijing, China). Chloroform, bromothymol blue, acetonitrile, xylene, glacial acetic acid, anhydrous sodium sulfate and sodium bicarbonate were purchased from Chengdu Kelong Chemical Reagent Factory (Chengdu, China). *Chuanxiong* samples were collected from Sichuan, Yunnan, Jiangsu, Jiangxi, Gansu, Guizhou and Jilin, and were identified by Professor Yuying Ma of Chengdu University of TCM, Chengdu, China. All samples from different *chuanxiong* producing areas were preserved in the germplasm nursery. In all, a total of 16 samples were analyzed, as shown in Table 1.

Table 1: *Chuanxiong* samples used, and their sources

No.	Place of origin	No.	Place of origin
1	Dujiangyan, Sichuan	9	Chongzhou, Sichuan
2	Dujiangyan, Sichuan	10	Chongzhou, Sichuan
3	Dujiangyan, Sichuan	11	Nantong, Jiangsu
4	Pengshan, Sichuan	12	Yulong, Yunnan
5	Pengshan, Sichuan	13	Heqing, Yunnan
6	Xindu, Sichuan	14	Heqing, Yunnan
7	Pixian, Sichuan	15	Ruichang, Jiangxi
8	Shifang, Sichuan	16	Huating, Gansu

Determination of volatile oils

The volatile oil contents of the *chuanxiong* samples were determined according to the procedure outlined in Chinese Pharmacopoeia, 2015 edition, volume IV.

Determination of total alkaloids

Total alkaloids were determined according to the method outlined in Chinese Pharmacopoeia, 2015 edition, volume IV.

Determination of total ferulic acid content

Instrumentation and separation conditions

Total ferulic acid content was determined with high performance liquid chromatography (HPLC) performed on Waters 2695 HPLC instrument (Waters, UK) equipped with a diode array detector (DAD) detector, and a Kromasil C18 column (4.6 mm × 250 mm, 5 µm).

The mobile phase was composed of acetonitrile (A) and 1 % glacial acetic acid aqueous (B) with a volume ratio of 18:82. The flow rate was 1.0mL/min, and injection volume was 10 µL. The temperature of column was set at 30 ℃. Ferulic acid was monitored at a wavelength of 320nm [3].

Sample preparation

Chuanxiong was ground into powder, and 0.5 g of the powder was dissolved in 25 mL of a solution comprised of methanol and 2 % sodium bicarbonate (the volume ratio was 95:5). Ultrasonic extraction was carried out for 100 min. The solution was cooled to room temperature and loss in volume was made up for with 2 % sodium bicarbonate. The mixture was filtered through a 0.45µm filter before injection.

Preparation of standard solutions

An appropriate amount of ferulic acid was weighed accurately and dissolved in methanol - glacial acetic acid (99:1 v:v) solution to yield a ferulic acid concentration of 26.56 µg/mL.

Validation of method and sample determination

The HPLC method was employed and the methodology was evaluated for linearity, precision, repeatability, stability and recovery. Five different volumes of the standard solution (2, 6, 10, 14 and 18 µL) were subjected to HPLC analysis. A linear regression equation was drawn with the injection volume as *x*-axis and the peak area as *y*-axis, and it exhibited good linearity. The precision of the method was evaluated by determining the same sample 6 times, while

repeatability was obtained by analyzing 6 replicates of the same sample. Stability was tested at several time points (1, 2, 4, and 8 h), and the ferulic acid was stable for 8 h. The recovery was analyzed by spiking known quantity of the standard into 6 *chuanxiong* samples extracted according to sample preparation. The spiked samples were analysed with HPLC. Total ferulic acid was determined in all samples.

GC -MS analysis of volatile oil, instrumentation and GC -MS conditions

The GC-MS analysis was performed with HP-6890/5973 GC-MS instrument with hydrogen Flame Ionization detector (FID) and an HP-1 MS capillary column (60 m ×0.25 mm ×0.25μm). The mass spectrometer conditions were similar to literature methods, the carrier gas was Helium, and the flow rate was set as 1.8mL/min. The split ratio was 1:20. The mass spectrometer was fitted with an EI source operated at 30 eV with a source temperature of 260 °C [3]. Mass spectra were recorded in the full-scan acquisition mode in the range of *m/z* 20 - 450 amu.

Sample preparation

Chuanxiong powder (no.7) was precisely weighed (100g) and steam-distilled with 300 mL of water for 6 h in a round-bottom flask, made up to 2 mL with xylene, and dried with anhydrous sodium sulfate. Then, 0.15 mL of internal standard solution was thoroughly mixed with 0.15 mL of volatile oil extraction solution.

Preparation of Standard Solution

The reference standard [(+) – camphor] was weighed and dissolved in xylene to yield a concentration of 2.012 mg/mL.

GC fingerprint of volatile oil, instrumentation and separation conditions

The GC fingerprint determinations were performed using a Waters GC -2014 instrument (Shimadzu, Japan) equipped with a hydrogen FID and a SUPLCO DB-5 capillary column (30 m × 0.25 mm × 0.25 μm). N_2 was used as the carrier gas and the flow rate was 1 mL/min. The temperature of detector and the vaporization chamber were 260°C. The split ratio was 1:20, and injection volume was 1 μL. The GC temperature was programmed as follows: 60 to 110°C at a rate of 2.5 °C/min, and then to 160°C at a rate of 10 °C/min, and finally to 190 °C at a rate of 1.5 °C/min [3].

Sample preparation

The method of sample preparation for GC fingerprint was identical to that described in GC-MS analysis of volatile oil.

Method validation

The GC methodology was assessed for precision, repeatability and stability. The precision was evaluated by analyzing the same sample 6 times, while repeatability was obtained by determining 6 replicates of same sample. The stability was tested at several time points (1, 2, 4, and 8 h), and the sample was found to stable for 8 h.

RESULTS

Volatile oil contents

There were no significant differences in contents of volatile oil in *chuanxiong* samples from different producing areas, except the sample from Jiangxi. The levels of volatile oil in some of the *chuanxiong* samples were increased after transplantation to the germplasm nursery in Dujiangyan. These results are shown in Table 2.

Total alkaloid contents

As shown in Table 2, there were no significant differences in the contents of total alkaloids of *chuanxiong* from the various sources.

Total ferulic acid levels

The chromatograms of standard ferulic acid and ferulic acid in *chuanxiong* samples are shown in Figure 1, and the contents of ferulic acid are presented in Table 2.

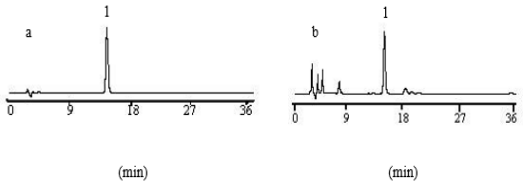

Figure 1: Chromatogram of ferulic acid standard (a), and *chuanxiong* sample (b).

The results in Table 2 show that there were no significant differences in the levels of the main components among the *chuanxiong* samples, except for samples 4, 11 and 15. In most of the *chuanxiong* samples, the levels of volatile oil, total alkaloids and total ferulic acid were increased after transplantation to the germplasm nursery in Dujiangyan, Sichuan.

Table 2: Levels of volatile oil, total alkaloids and total ferulic acid in *Chuanxiong* samples

S/no.	*Chuanxiong* from its place of origin			*Chuanxiong* transplanted to germplasm nursery		
	Volatile oil (%)	*Total alkaloids (%)*	*Total ferulic acid (%)*	*Volatile oil (%)*	*Total alkaloids (%)*	*Total ferulic acid (%)*
1	0.4050	0.2040	0.1745	0.2500	0.2799	0.2135
2	0.3750	0.1946	0.1251	0.3000	0.3234	0.2318
3	0.3750	0.3410	0.1145	0.4700	0.1805	0.1577
4	0.2650	0.1239	0.2475	0.2400	0.1764	0.2506
5	0.3800	0.2545	0.2300	0.4200	0.3182	0.1857
6	0.3900	0.1436	0.1874	0.4200	0.2252	0.1983
7	0.4400	0.2142	0.2100	0.2200	0.2761	0.2052
8	0.3700	0.2692	0.0460	0.3500	0.1721	0.1848
9	0.4650	0.1209	0.1033	0.2700	0.1806	0.2042
10	0.2900	0.1518	0.1510	0.2700	0.2318	0.1948
11	0.2550	0.1181	0.0571	0.3200	0.1636	0.1602
12	0.4550	0.4143	0.2083			
13	0.6200	0.2084	0.3450			
14	0.4350	0.1308	0.2241			
15	0.1850	0.0849	0.0564			
16	0.4650	0.2460	0.2352			

Results from GC-MS analysis

The total ion flow graph of the samples determined with GC-MS is shown in Figure 2. The major components of the volatile oils were identified through comparison with literature. The results show that phthalides were the main components of *chuanxiong* volatile oil, accounting for about 60 -70 % of the oil. The other components were terpenoids, aliphatic hydrocarbons and a small amount of organic acids. The results of analysis of chemical composition are shown in Table 3.

GC fingerprint results

The chromatograms are shown in Figure 3 and Figure 4, respectively. In view of the fact that the different chemical components have different contributions to the chemical base of *chuanxiong*, only the chromatogram from 9.01 min to 43 min were analyzed. The data were imported into the "Computer Aided Similarity Evaluation System" software for analysis of similarities in the chromatogram from 9.01 min to 43 min. The results are shown in Table 4.

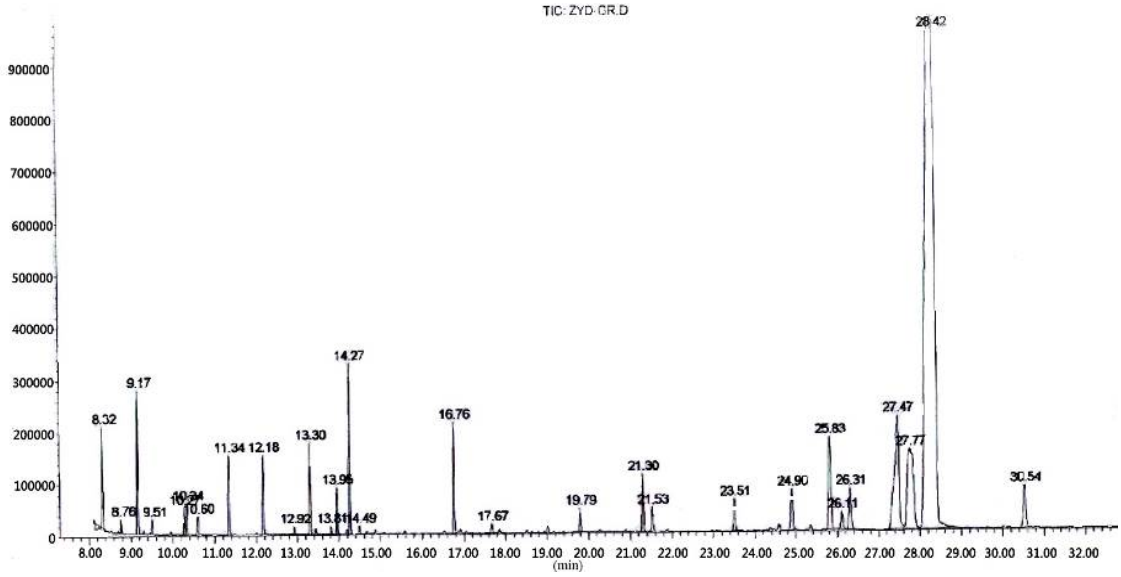

Figure 2: Total ion flow graph of volatile oils in *chuanxiong* samples

Table 3: Major components of volatile oils from the *chuanxiong* samples

S/no.	Retention time (min)	Compound	Molecular formula	Relative peak area
1	8.32	α-Pinene	$C_{15}H_{24}$	0.99
2	8.76	Phenol	C_6H_5OH	0.18
3	9.17	Terpene	$C_{10}H_{16}$	1.88
4	9.51	Myrcene	$C_{10}H_{16}$	0.23
5	10.27	δ-4-Carene	$C_{10}H_{16}$	0.39
6	10.34	P-Cymene	$C_{10}H_{14}$	0.44
7	10.60	β-Phellandrene	$C_{10}H_{16}$	0.27
8	11.34	γ-Terpinene	$C_{10}H_{16}$	1.16
9	12.18	α-Terpinolene	$C_{10}H_{16}$	1.15
10	12.92	1-methyl-4-isopropyl-2-cyclohexen-1-ol	$C_{10}H_{18}O$	0.12
11	13.30	(+)-Camphor	$C_{10}H_{16}O$	1.35
12	13.81	Amylbenzene	$C_{11}H_{16}$	0.12
13	13.95	5-Ethyl-3-ynyl-undecane	$C_{11}H_{18}$	0.65
14	14.27	Terpineol-4	$C_{10}H_{18}O$	2.30
15	14.49	α- Terpinene	$C_{10}H_{16}$	0.10
16	16.76	4-hydroxy-2-methoxystyrene	$C_9H_{10}O_2$	1.39
17	17.67	Valerophenone	$C_{11}H_{14}O$	0.13
18	19.79	6-methyl-2-p-methylphenyl-heptane	$C_{15}H_{20}$	0.42
19	21.30	(+)-β-selinene	$C_{15}H_{24}$	1.11
20	21.53	Germacrene	$C_{15}H_{24}$	0.50
21	23.51	Spartanol（Spaihulenol）	$C_{15}H_{24}O$	0.72
22	24.90	Butylphthalide	$C_{12}H_{14}O_2$	1.15
23	25.83	Butylidene phthalide	$C_{12}H_{12}O_2$	2.70
24	26.31	(E,E)-1,3,5-undecatriene	$C_{11}H_{18}$	1.35
25	27.47	Senkyunolide A	$C_{12}H_{16}O_2$	6.90
26	27.77	neocindilide	$C_{12}H_{18}O_2$	6.45
27	28.42	*Z*- Ligustilide	$C_{12}H_{14}O_2$	63.99
28	30.54	*E*- Ligustilide	$C_{12}H_{14}O_2$	1.34

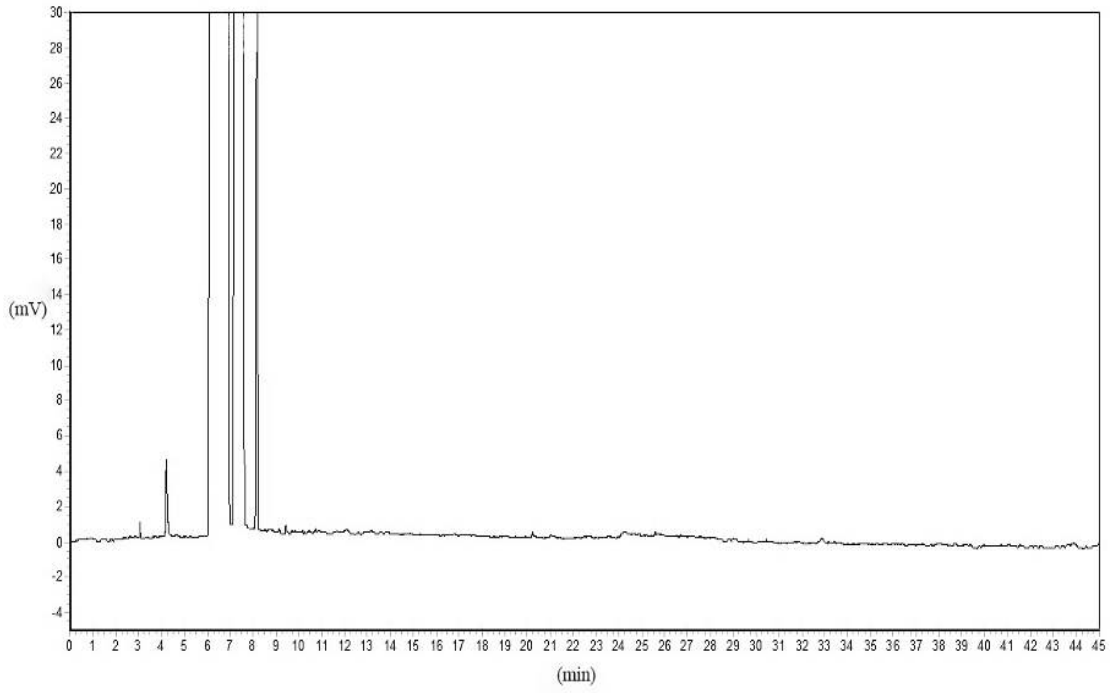

Figure 3: GC of blank solvent

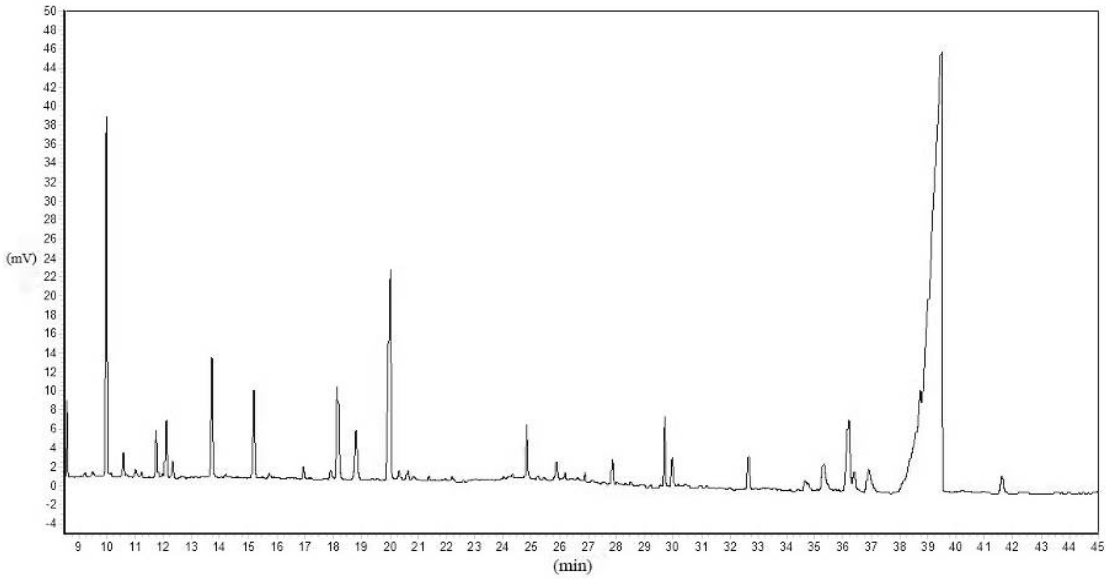

Figure 4: GC of test samples

Table 4: Results of similarity analysis of the chromatogram

No.	Similarity		No.	Similarity
	Chuanxiong from place of origin	***Chuanxiong transplanted to germplasm nursery***		
1	0.8505	0.9204	12	0.8401
2	0.9329	0.9534	13	0.9039
3	0.9258	0.8733	14	0.9484
4	0.8294	0.9383	15	0.9235
5	0.8968	0.9362	16	0.8122
6	0.8924	0.9589		
7	0.9618	0.9512		
8	0.9372	0.9203		
9	0.9810	0.9440		
10	0.9673	0.9003		
11	0.8500	0.9529		

The range of similarity of *chuanxiong* from different places of origin was 0.8122 - 0.9810. The similarities of *chuanxiong* from different places in Sichuan province were not all greater than 0.9, which indicates differences in compositions and proportions of components. The similarities of *chuanxiong* from Yunnan and Gansu province were less than those of *chuanxiong* from Sichuan province, indicating that there were large differences in composition and proportion of components. There were smaller differences among the chemical compositions of volatile oils in *chuanxiong* samples from different places in Sichuan. Moreover, there were some differences in chemical composition of volatile oils in different *chuanxiong* germplasm resources. Following transplantation to the germplasm nursery, almost all the similarities were increased. These results are shown in Figure 5.

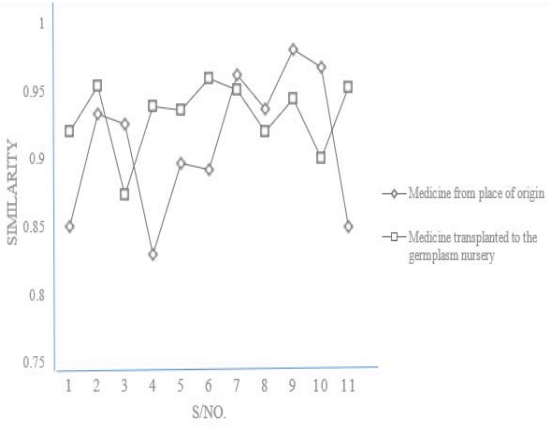

Figure 5: Graphical representation of similarities between volatile oil of *chuanxiong* from original planting areas and volatile oil from *chuanxiong* transplanted to germplasm nursery

DISCUSSION

It has been reported that *chuanxiong* promotes blood circulation, activates *qi, expels wind* and alleviates pain [1]. Research has shown that *chuanxiong* exerts several pharmacological properties such as sedation and analgesia [4-6], anti-freezing [7,8], cardiovascular and cerebrovascular protection [9], and anti-inflammatory effect [10,11]. *Chuanxiong* is widely used in the treatment of coronary heart disease (CHD), angina pectoris [12], ischemic brain injury [13] and migraine. Studies [14-16] show that the traditional blood-activating and pain-relieving effects of *chuanxiong* are due to the pharmacological properties of alkaloids such as tetramethylpyrazine (TMP), phenolic acids (e.g. ferulic acid) and phthalides (e.g. ligustilide).

However, it is difficult to accurately evaluate the quality of *chuanxiong* through the determination of ferulic acid, TMP, and ligustilide, either separately or together. In addition, it has been reported that ferulic acid content increases with storage time because of the hydrolysis of ferulic acid conifers during storage. Therefore, it is not appropriate to control the quality of *chuanxiong* through determination of free ferulic acid alone. This gave rise to the concept of total ferulic acid which was developed to solve this problem in the determination of ferulic acid in *Angelica sinensis* [17]. Thus, in this study, based on Chinese Pharmacopoeia, preliminary experiments [18] and literature, the content of volatile oil was determined, and the contents of total alkaloids and total ferulic acid were added to comprehensively evaluate the quality *chuanxiong*.

According to Materia medica, the traditional and authentic production area of *chuanxiong* was Dujiangyan (named Guan county in ancient times) in Sichuan province. In the record of Materia medica, *xiongqiong* was also planted in other places apart from Suchuan, and named after its locality. For example, it is named *fuxiong* when produced in “Fuzhou” (now Fuzhou, Jiangxi, China), while the product from Yunnan, China is called *lixiong* or *yunxiong*. Scholars have studied the original plants, chromosomes, and chemical composition of *chuanxiong* from different producing areas based on Materia medica, and the results showed differences in the germplasm resources of *chuanxiong*. The present study compared *chuanxiong* from Dujiangyan and *chuanxiong* from other provinces. Besides, changes in volatile oil and contents of major components of *chuanxiong* produced in other places in Sichuan and introduced from Sichuan, were compared after transplantation to the germplasm nursery in Dujiangyan.

CONCLUSION

This study provides a comprehensive method for quality evaluation of *L. Chuanxiong* Hort. from different producing areas and comparison of different germplasm resources. The results show that the method of GC analysis for determination of volatile oils and main components of *chuanxiong* is accurate and stable for comparison of quality of *chuanxiong* from different producing areas. The results indicate that an authentic production area is beneficial to the generation of stable germplasm resources. The quality of *chuanxiong* produced in Dujiangyan was better than the quality of *chuanxiong* from other areas, and it may produce high efficacy in clinical application.

This study also provides theoretical basis for validation of authenticity of *chuanxiong* from Dujiangyan. Combined with previous research on the operational regulations of standardized production technology standards of *chuanxiong*, this study may lead to improved industrial advantage in the cultivation of *chuanxiong* in Dujiangyan.

DECLARATIONS

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Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them. Ling Chen and Jia Hou contributed equally to this work.

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