LJMU Research Online

Huang, S, Chen, J, Li, W, Song, S, Li, X, Yu, H, Long, F, Chen, R, Bao, X, Chan, K and Lu, G

Comparison of Volatile Compositions among Four Related Ligusticum chuanxiong Herbs by HS-SPME-GC-MS

http://researchonline.ljmu.ac.uk/id/eprint/19240/

Article

Citation (please note it is advisable to refer to the publisher's version if you intend to cite from this work)

Huang, S, Chen, J, Li, W, Song, S, Li, X, Yu, H, Long, F, Chen, R, Bao, X, Chan, K and Lu, G (2023) Comparison of Volatile Compositions among Four Related Ligusticum chuanxiong Herbs by HS-SPME-GC-MS. Processes, 11 (1).

processes

MDPI

Article

Comparison of Volatile Compositions among Four Related *Ligusticum chuanxiong* Herbs by HS-SPME-GC-MS

Shiwei Huang [1], Jiamei Chen [1], Wan Li [1], Shanghong Song [1], Xiaoxue Li [1], Han Yu [1], Fei Long [1,*], Rong Chen [2], Xiaoming Bao [3], Kelvin Chan [4,5,*] and Guanghua Lu [1,*]

[1] State Key Laboratory of Southwestern Chinese Medicine Resources, School of Pharmacy, Chengdu University of Traditional Chinese Medicine, Chengdu 611137, China
[2] School of Ethnic Medicine, Chengdu University of Traditional Chinese Medicine, Chengdu 611137, China
[3] Shimadzu (China) Company Limited, Chengdu 610023, China
[4] Centre for Natural Products Discovery, School of Pharmacy & Biomolecular Sciences, Liverpool John Moores University, Liverpool L3 3AF, UK
[5] NICM Health Research Institute, Western Sydney University, Sydney, NSW 1797, Australia
* Correspondence: longfei@cdutcm.edu.cn (F.L.); k.k.chan@ljmu.ac.uk (K.C.); lugh@cdutcm.edu.cn (G.L.)

Abstract: Chuanxiong (CX, *Ligusticum chuanxiong*), Japanese Chuanxiong (JCX, *Cnidium officinale*), Fuxiong (FX, *Ligusticum sinense* 'Fuxiong'), and Jinxiong (JX, *Ligusticum sinense* 'Jinxiong') are aromatic herbs used in China, Japan, and other regions. Their morphology and aromatic odor are similar, resulting in confused and mixed uses. This study compares the volatile compositions of these herbs for defining their medical uses. Headspace solid-phase microextraction–gas chromatography–triple quadrupole–mass spectrometry was employed to separate, identify, and quantify the compounds in the volatile gas of the four herbs. A total of 128 volatile compounds were identified and quantified in 23 these herbal samples. The sums of 106, 115, 116, and 120 compounds were detected in the volatile gas of CX, JCX, FX, and JX, with the mean contents of 4.80, 7.12, 7.67, and 12.0 μg/g, respectively. Types and contents of the main compounds were found to be different in the volatile gas of these herbs. The orthogonal partial least squares discriminant analysis and hierarchical clustering analysis showed the four herbs located in different confined areas or clusters. It is concluded that the volatile compositions in the four herbs are generally similar, but the contents of main volatile compounds are different. These herbs should be clearly differentiated in medical use.

Keywords: *Ligusticum chuanxiong*; *Cnidium officinale*; *Ligusticum sinense* 'Fuxiong'; *Ligusticum sinense* 'Jinxiong'; volatile composition; HS-SPME-GC-MS

check for updates

Citation: Huang, S.; Chen, J.; Li, W.; Song, S.; Li, X.; Yu, H.; Long, F.; Chen, R.; Bao, X.; Chan, K.; et al. Comparison of Volatile Compositions among Four Related *Ligusticum chuanxiong* Herbs by HS-SPME-GC-MS. *Processes* **2023**, *11*, 196. https://doi.org/10.3390/pr11010196

Academic Editors: Elwira Sieniawska and Alina Pyka-Pająk

Received: 16 November 2022
Revised: 27 December 2022
Accepted: 3 January 2023
Published: 7 January 2023

1. Introduction

Chuanxiong (rhizome of *Ligusticum chuanxiong* Hort., or *Ligusticum sinense* 'Chuanxiong', CX) and Japanese Chuanxiong (rhizome of *Cnidium officinale* Makino, JCX) are widely used herbs in China and Japan. Although the name of JCX is "Senkyu" in Japan, its transliteration name is the same as the Chinese "Chuanxiong" [1]. They play an almost identical medical role in prescription or proprietary medicine in China and Japan. CX is cultivated mainly in Sichuan Province in China. JCX was introduced to Japan from China in the Edo period (around the 17th century), but it is presently introduced back to China from Japan and cultivated in Sichuan Province and Jilin Province in China [2–5]. Thus, these two herbs are easily confused. Two other related herbs are Fuxiong (rhizome of *Ligusticum sinense* 'Fuxiong', FX) and Jinxiong (rhizome of *Ligusticum sinense* 'Jinxiong', JX). FX is cultivated in Jiangxi Province and Hubei Province in China for its healthcare function, which is used mainly for tea with the synonyms name "Chaxiong" [6]. JX is cultivated mainly in Yunnan, Guizhou, Shaanxi Province in China [7]. FX and JX are also denoted as Chuanxiong, and there exists confusion regarding their medical uses. Moreover, official uses of these herbs are rather limited in different countries.

Aroma is known as a complex mixture of many species–specific volatile compounds and varies with plant species [8], which is one of the main indicators to assess the authenticity and quality of these aromatic herbs. In normal practice, strong aromatic aromas are indicators of good quality for the four herbs [9]. However, their unique aromatic smells are not identical and are difficult to distinguish. The known bioactive compounds in these herbs are Z-ligustilide, senkyunolide A, and butylphthalide. Volatile flavor compounds in plants are affected by many factors: species or variety, climatic factors, cultural practices, and harvest and postharvest processing [10]. Therefore, it is useful to compare their volatile compositions for defining the medical uses of CX, JCX, FX, and JX.

To analyse the bioactive compounds, steam distillation, organic solvent extraction, and supercritical carbon dioxide extraction, is usually used to extract volatile oils from these herbs; then, GC or GC-MS techniques are employed to separate, identify, or quantify the levels of volatile compounds [11–13]. However, the chemical structures of volatile compounds are easily oxidized, decomposed, and/or transformed during the heating of volatile oils in extraction. Natural and real volatile compounds are not easily obtained in these herbs [14]. Recently, by application of the headspace solid-phase microextraction (HS-SPME) technique, the volatile gas in herbal powder is easily collected under a relatively low temperature of heating. This volatile gas is injected into the GC to be separated and detected. Therefore, the types and amounts of natural and real volatile compounds in aromatic herbs can be obtained [15–17].

In the present study, 23 samples for the four aromatic herbs, namely CX, JCX, FX, and JX, were collected from different cultivation areas in China. A total of 154 chemical standards were used to identify and quantify the levels of compounds in the volatile gas. The sum of 128 kinds of volatile compounds was identified, quantified, and analyzed in these herbs by the headspace solid-phase microextraction–gas chromatography–triple quadrupole–mass spectrometry (HS-SPME-GC-QQQ-MS) combined with the Shimadzu Off-flavor-TQ-MS database, orthogonal partial least squares discriminant analysis (OPLS-DA), and hierarchical clustering analysis (HCA) statistical methods. The types, contents, and chemical structures of volatile compounds were compared. The present study provides scientific data for the identities of volatile compounds to distinguish the suitable uses of these related herbs.

2. Materials and Methods

2.1. Instrumentation

Gas chromatograph–triple quadrupole–mass spectrometer (GCMS-TQ8050nci, Shimadzu, Kyoto, Japan) equipped with a PAL SHIMADZU auto-sampler (PLA-RTC-120, Struthers Analytical Instruments Ltd., Zwingen, Switzerland) was employed to separate and detect the volatile components. An off-line analysis database (Off-flavor-database, Shimadzu, Kyoto, Japan) was used to identify and quantify the chemical compounds.

2.2. Materials and Chemicals

A total of twenty-three herbal samples were collected from different cultivation areas in China during their respective harvesting periods (Table 1). Ten CX samples were collected in Dujiangyan, Pengzhou, and Shifang of Sichuan from May 2020 to June 2021. Three JCX samples were collected in Pengzhou of Sichuan in October 2014, which were introduced from Japan and cultivated. Seven FX samples were collected at Yushan of Hubei Province or Jiujiang of Jiangxi Province in July or October 2021. Three JX samples were collected in Tengchong of Yunnan province in November 2021. These herbal samples were authenticated by Prof. Guanghua Lu in the School of Pharmacy, Chengdu University of Traditional Chinese Medicine. All samples were dried, sealed, and stored in a cool condition in this institution. The chemical standards (purity > 95%) were purchased from Chengdu Pusi Biotechnology Co., Ltd. (Pusi Biotechnology Co., Ltd., Chengdu, China).

Table 1. Information of Herb Samples.

No.	Herbal Name	Plant Species	Collection Area	Collection Year/Month
1	Chuanxiong	*Ligusticum chuanxiong* Hort.	Dujiangyan, Sichuan, China	2020.05
2	Chuanxiong	*Ligusticum chuanxiong* Hort.	Dujiangyan, Sichuan, China	2020.05
3	Chuanxiong	*Ligusticum chuanxiong* Hort.	Dujiangyan, Sichuan, China	2021.06
4	Chuanxiong	*Ligusticum chuanxiong* Hort.	Dujiangyan, Sichuan, China	2021.06
5	Chuanxiong	*Ligusticum chuanxiong* Hort.	Dujiangyan, Sichuan, China	2021.06
6	Chuanxiong	*Ligusticum chuanxiong* Hort.	Dujiangyan, Sichuan, China	2021.06
7	Chuanxiong	*Ligusticum chuanxiong* Hort.	Dujiangyan, Sichuan, China	2021.06
8	Chuanxiong	*Ligusticum chuanxiong* Hort.	Pengzhou, Sichuan, China	2020.05
9	Chuanxiong	*Ligusticum chuanxiong* Hort.	Pengzhou, Sichuan, China	2020.05
10	Chuanxiong	*Ligusticum chuanxiong* Hort.	Shifang, Sichuan, China	2020.05
11	Japanese Chuanxiong	*Cnidium officinale* Makino	Pengzhou, Sichuan, China	2014.10
12	Japanese Chuanxiong	*Cnidium officinale* Makino	Pengzhou, Sichuan, China	2014.10
13	Japanese Chuanxiong	*Cnidium officinale* Makino	Pengzhou, Sichuan, China	2014.10
14	Fuxiong	*Ligusticum sinense* 'Fuxiong'	Jiujiang, Jiangxi, China	2021.07
15	Fuxiong	*Ligusticum sinense* 'Fuxiong'	Jiujiang, Jiangxi, China	2021.07
16	Fuxiong	*Ligusticum sinense* 'Fuxiong'	Jiujiang, Jiangxi, China	2021.07
17	Fuxiong	*Ligusticum sinense* 'Fuxiong'	Jiujiang, Jiangxi, China	2021.07
18	Fuxiong	*Ligusticum sinense* 'Fuxiong'	Yushan, Hubei, China	2021.10
19	Fuxiong	*Ligusticum sinense* 'Fuxiong'	Yushan, Hubei, China	2021.10
20	Fuxiong	*Ligusticum sinense* 'Fuxiong'	Yushan, Hubei, China	2021.10
21	Jinxiong	*Ligusticum sinense* 'Jinxiong'	Tengchong, Yunnan, China	2021.11
22	Jinxiong	*Ligusticum sinense* 'Jinxiong'	Tengchong, Yunnan, China	2021.11
23	Jinxiong	*Ligusticum sinense* 'Jinxiong'	Tengchong, Yunnan, China	2021.11

2.3. Preparation of Standard Solution

The chemical standard (10.00 mg) was accurately weighed and transferred into a 10 mL volumetric flask. Methanol was added to prepare as stock solution with a concentration of 1.00 mg/mL. Then, 50 μL of the stock solution and 950 μL methanol were added into a small bottle to obtain a single standard solution with a concentration of 0.05 mg/mL. For the preparation of the mixed standard solution, each standard stock solution was added into a volumetric flask, and then methanol was added. The concentration of each standard was 0.05 mg/mL.

2.4. Regression Method and Correction

The gradient standard solutions were prepared in different concentrations of 1 pg/μL, 10 pg/μL, 100 pg/μL, 1000 pg/μL, and 10,000 pg/μL. Of each standard solution, 1 μL was injected into GC chromatographer to detect the peak areas (μV·s). The regression equation was calculated by the peak area (μV·s) and the concentrations (pg/μL) of standard solutions. Meanwhile, the retention times of 154 standard compounds were corrected with n-alkanes (C9–C30). The regression equation was corrected by three internal standards, i.e., 4-bromofluorobenzene, 1,2-dichlorobenzene-d4, and acenaphthene-d10. These regression equations were used to calculate the contents of volatile compounds in samples (Table S1).

2.5. Preparation of n-alkanes Solution (C9–C30) and Internal Standards Solution

Twenty-two n-alkanes solution (C9–C30) were prepared with the concentration of 0.1 μg/mL. Three internal standards solutions were prepared with the concentration of 10 μg/mL. Of these solutions, 1 μL was injected into GC chromatographer for GC-MS analysis to correct the retention time and obtain the regression equation.

2.6. Preparation of Sample for GC-MS Analysis

Representative samples were cut into smaller pieces and further ground into powder and passed through a 10-mesh sieve.

The herbal sample powder (0.1 g) was accurately weighed and transferred into the headspace injection vial. The headspace injection vial was then equilibrated in the oven of auto-sampler for 40 min at 50 °C. The solid-phase extraction head was inserted into the headspace injection vial without touching the sample powder, and absorbed volatile composition to saturation at 50 °C was attained. Then, the solid-phase extraction head was quickly transferred into the inlet of GC-MS system and desorbed for 2 min at 250 °C for the GC-MS analysis. Sample duplicates were prepared and analyzed by the same analytical procedures.

2.7. Gas Chromatography Conditions

The volatile components were separated by an Inert Cap Pure-WAX capillary column (0.25 mm × 30 m, 0.25 μm). Injection was operated in split mode (5:1) at 250 °C. Helium (99.99% purity) was used as the carrier gas, with the front inlet purge flow rate of 3.0 mL/min and a constant gas flow rate through the column of 1.46 mL/min. Control mode was pressure control with pressure 83.5 Kpa. The program of oven temperature was initiated at 50 °C for 5 min, then ramped up to 250 °C at a rate of 10 °C/min and held for 15 min.

2.8. Mass Spectrometry Conditions

Mass spectra in electron impact ionization mode were recorded at ionization energy of 70 eV. The collision gas was argon. Temperatures of MS source and quadrupole were set to 200 and 250 °C, respectively. The solvent delay time was 1.19 min. The monitoring mode of mass spectrometry was multiple reaction monitoring (Table S1).

2.9. Data Analysis

The GC-MS data were analyzed by GC-MS solution software (version 4.52, Shimadzu, Kyoto, Japan). The collected data were input into SIMCA 14.1 (Umetrics, Umea, Sweden) for OPLS-DA and HCA.

For the identification of chemical compounds, the retention time of the peaks of testing compounds in the sample was firstly corrected by the data of 22 types of n-alkanes (C9–C30). Then, the retention time, retention index, and MS data, including precursor ions and product ions of specific peak, were compared with those of the 154 standard compounds in the database (Table S1).

For the semi-quantification of volatile compounds in the volatile gas, the calibrating curves/regression equations of the 154 standard compounds were corrected by three internal standards, i.e., 4-bromofluorobenzene, 1,2-dichlorobenzene-d4, and acenaphthene-d10. The content for individual volatile compounds was calculated by the regression equation. Meanwhile, a quantifier ion and two qualifier ions were used for quantitation purpose (Tables S1 and S2).

3. Results

3.1. Optimization of Analytical Method

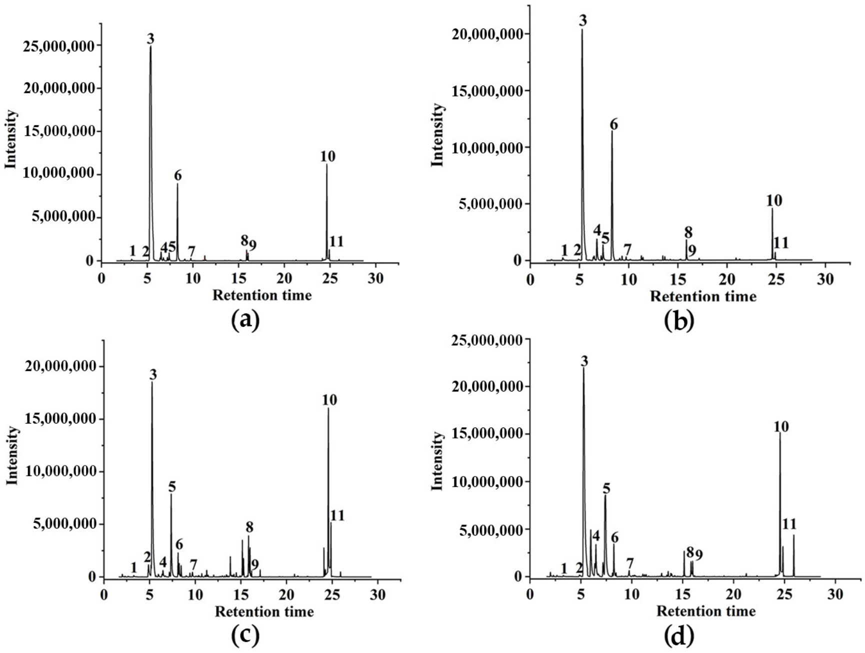
The present method was developed on the basis of the reference [18]. The main peaks were well separated on the GC chromatograms, and these volatile compounds could be detected under the HS-SPME-GC-MS condition. Various factors were further evaluated and chosen.

For the choice of suitable sample amount, 0.1, 0.2, 0.3, 0.4, and 0.5 g CX sample powder, respectively, was placed into the headspace injection vial, and then the contents of compounds in volatile gas were analyzed. Although the sum of detected compounds was found to have increased with the improvement of sample amount, the contents of some compounds, e.g., ligustilide, senkyunolide A, and 3-butylidenephthalide, were larger in the sample with the small amount (0.1 g). Hence, the amount of 0.1 g was chosen as the sample amount in this study.

For the selection of SPME fiber, the fibers of PDMS, PDMS-DVB, PA, and DVB-CAR-PDMS were evaluated. PDMS fiber is used mainly to analyze samples with non-polar volatile compounds. PDMS-DVB is usually used to detect alcohols and amines in samples with polar compounds. PA is often used to detect semi-volatile components (phenols) in samples. DVB-CAR-PDMS fiber contains a composite material (ARR15-DVB/C-WR-120/20) and is capable of adsorbing compounds of various chemical properties, especially the analysis of a wide range of C3–C20 compounds. Because aroma is known as a complex mixture of many species–specific volatile compounds and varies with plant species [8], DVB-CAR-PDMS fiber is suitable for complex constituents and was chosen in this study.

3.2. Identification of Compounds in Volatile Gas

A total of 128 compounds were unequivocally identified on the GC chromatograms of the four herbs by comparing the retention time, retention index, and the MS data of precursor ions and product ions with the 154 standard compounds. Owing to the unavailability of authentic compounds, some main peaks could not be identified on the GC-MS chromatograms (Figure 1). These mass spectra of these unknown compounds were compared in the NIST 17 database. These peaks were tentatively assigned as the compounds with high similarity of mass spectrum, namely peak 1: (3R,5R)-5-methyl-3-prop-1-en-2-ylcyclohexene (86%); peak 2: ethylidene fluoride (91%); peak 5: terpinolene (94%); peak 6: vinyl acrylate (92%); peak 7: tropylium iodide (68%); peak 9: β-selinene (96%); peak 10: 3-isobutylidenephthalide (92%); and peak 13: 1-methyl-1H-1,2,3-benzotriazol-7-amine (70%). For the unambiguous identification of these compounds, further studies are required by using authentic compounds.



(a) (b) (c) (d)

Figure 1. The TIC chromatograms of GC-MS in the four herbs. (**a**) Chuanxiong (*Ligusticum chuanxiong*); (**b**) Japanese Chuanxiong (*Cnidium officinale*); (**c**) Fuxiong (*Ligusticum sinense* 'Fuxiong'); (**d**) Jinxiong *(Ligusticum sinense* 'Jinxiong'). 1: (3R,5R)-5-methyl-3-prop-1-en-2-ylcyclohexene; 2: Ethylidene fluoride; 3: 2-Heptanone; 4: Limonene; 5: Terpinolene; 6: Vinyl acrylate; 7: Tropylium iodide; 8: N-valeric acid; 9: β-selinene; 10: 3-isobutylidenephthalide; 11: Ligustilide; 12: Senkyunolide A; and 13: 1-Methyl-1H-1,2,3-benzotriazol-7-amine.

3.3. Correct of Retention Time and Regression Equation

In order to accurately recognize and identify the peaks, twenty-two n-alkanes (C9–C30) were chosen to correct the retention time of these peaks. For the accurate quantitation of compounds in volatile gas of these herbs, three internal standards, such as 4-bromofluorobenzene, 1,2-dichlorobenzene-d4, and acenaphthene-d10, were used to correct the regression equations. The three internal standards possess the advantages of stable chemical structure, symmetric peaks, and appropriate retention times on the GC chromatogram.

3.4. Method Validation

In this analytical method, sample powder (0.1 g) was placed into the headspace injection vial, and the next analytical procedures—such as the production of volatile gas, the absorption of solid phase extraction head, and the desorption from this head—were conducted in the analytical system. Thus, the repeatability was evaluated by the six replicated analysis of sample powder. The RSD of the amounts for these compounds ranged from 1.36% to 9.65% (n = 55), indicating that the method was reproducible.

3.5. Comparing the Types of Volatile Compounds in the Four Herbs

In the study, a total of 23 herbal samples were analyzed by the HS-SPME-GC-QQQ-MS method. A total of 154 chemical standards were used to identify the volatile compounds in these samples by retention time, MS data, etc. (Table S1). The sum of 128 volatile compounds were found in the four herbs (Table 2). There were 106, 115, 116, and 120 compounds detected in the volatile gas of CX, JCX, FX, and JX, respectively. After comparing these compounds in various herbs, it was found that there were 97 volatile compounds commonly existing in the four herbs, which accounts for 75.8% of the total 128 detected compounds. The GC-MS chromatograms of the four herbs were generally similar (Figure 1). The results indicated that the volatile composition and chemical properties of the four related herbs were generally similar.

Table 2. The names and contents of compounds in the volatile gas of the four herbs.

No.	**Component**	**Chuanxiong (ng/g, n = 10)**	**Japanese Chuanxiong (ng/g, n = 3)**	**Fuxiong (ng/g, n = 7)**	**Jinxiong (ng/g, n = 3)**
	Phthalides				
1	Ligustilide	1047 ± 276.2	800.7 ± 1.332	1715 ± 386.7	1533 ± 25.30
2	Senkyunolide A	850.8 ± 416.8	529.5 ± 39.86	1069 ± 149.6	1007 ± 7.554
3	Butylphthalide	56.70 ± 14.00	21.72 ± 1.612	71.70 ± 32.29	77.90 ± 0.550
4	3-Butylidenephthalide	25.41 ± 17.31	37.98 ± 6.219	312.2 ± 105.5	982.7 ± 6.463
	Terpenoids				
5	alpha-Pinene	797.7 ± 333.7	1447 ± 68.84	762.6 ± 166.1	936.1 ± 10.88
6	Limonene	188.9 ± 107.8	193.8 ± 65.49	293.5 ± 85.97	1107 ± 28.13
7	Linalool	117.5 ± 56.84	277.9 ± 5.390	245.1 ± 41.35	246.1± 9.239
8	beta-Pinene	75.29 ± 30.93	252.4 ± 119.4	1008 ± 159.4	274.6 ± 2.615
9	Verbenone	9.919 ± 4.070	+	2.038 ± 1.698	26.13 ± 0.689
10	Borneol	4.472 ± 1.625	43.91 ± 2.016	66.94 ± 20.76	63.26 ± 1.479
11	2-Methylisoborneol	4.405 ± 1.385	5.820 ± 0.734	23.11 ± 7.818	3.636 ± 0.287
12	alpha-Terpineol	3.200 ± 0.555	25.58 ± 3.900	28.03 ± 8.355	2.284 ± 0.085
13	Verbenol	+	21.78 ± 1.596	13.95 ± 4.250	22.63 ± 0.285
14	Geraniol	+	2.113 ± 0.257	2.592 ± 3.199	1.432 ± 0.123
15	Camphor	+	1.531 ± 0.027	1.763 ± 0.879	2.278 ± 0.207
16	L-Menthol	+	+	4.876 ± 2.253	+
17	alpha-Ionone	+	+	+	+
18	Eucalyptol	+	-	-	-
19	beta-Ionone	-	-	+	+
	Organic acids				
20	Ethylic acid	250.7 ± 82.80	239.6 ± 23.76	-	-
21	Butyric acid	151.5 ± 66.49	285.0 ± 11.39	183.9 ± 74.33	79.83 ± 0.570
22	Caproic acid	42.53 ± 17.83	217.4 ± 5.023	25.12 ± 12.55	9.235 ± 0.206
23	n-Valeric acid	30.69 ± 19.30	130.2 ± 23.11	49.25 ± 18.41	12.97 ± 0.693
24	Isovaleric acid	21.78 ± 9.769	36.77 ± 2.373	68.37 ± 9.079	41.17 ± 0.948
25	Phenylacetic acid	6.612 ± 0.235	6.398 ± 0.010	6.613 ± 0.300	6.570 ± 0.103
26	2-Methyl butyric acid	6.333 ± 2.966	16.15 ± 4.662	21.29 ± 5.477	7.130 ± 0.398
27	Propionic acid	6.281 ± 2.764	39.12 ± 1.442	36.31 ± 9.761	8.317 ± 0.217
28	Enanthic acid	5.437 ± 2.162	+	+	+
29	Isobutyric acid	4.645 ± 1.367	5.141 ± 0.627	14.37 ± 6.171	12.43 ± 1.100
30	Pelargonic acid	1.457 ± 0.521	3.758 ± 0.445	7.366 ± 3.543	11.52 ± 1.396

Table 2. *Cont.*

No.	Component	Chuanxiong (ng/g, n = 10)	Japanese Chuanxiong (ng/g, n = 3)	Fuxiong (ng/g, n = 7)	Jinxiong (ng/g, n = 3)
31	Isocaproic acid	+	2.235 ± 0.514	103.3 ± 53.46	63.95 ± 1.193
32	Capric acid	-	+	4.233 ± 2.612	1.918 ± 0.081
33	Lauric acid	-	+	1.354 ± 1.062	1.617 ± 0.002
34	Caprylic acid	-	-	4.347 ± 2.673	1.851 ± 0.036
	Ketones				
35	2-Undecanone	406.2 ± 289.9	1568 ± 8.220	529.0 ± 61.98	168.5 ± 1.170
36	2-Heptanone	19.27 ± 10.21	12.70 ± 3.520	6.445 ± 4.461	16.56 ± 2.181
37	2-Nonanone	6.463 ± 3.472	14.39 ± 2.878	10.68 ± 3.185	20.50 ± 1.459
38	5-Nonanone	1.846 ± 0.827	+	14.45 ± 7.659	23.43 ± 2.776
39	Benzophenone	+	48.14 ± 6.421	+	45.11 ± 0.650
40	Diacetyl	+	+	30.14 ± 7.288	66.72 ± 0.650
41	5-Hexene-2-one	+	+	2.686 ± 0.904	+
42	Acetophenone	+	+	2.032 ± 0.554	2.545 ± 0.061
43	Isophorone	+	+	+	1.237 ± 0.068
44	Benzyl acetone	+	+	+	+
45	3-Heptanone	+	+	+	+
46	Acetoin	-	-	-	846.4 ± 20.81
47	2-Hexanone	-	-	-	+
	Aromatics				
48	Toluene	246.0 ± 80.09	248.6 ± 114.4	42.95 ± 11.01	72.04 ± 2.430
49	alpha-Methylstyrene	34.16 ± 17.42	21.51 ± 5.808	83.37 ± 18.19	178.2 ± 1.415
50	Ethylbenzene	14.08 ± 7.531	+	+	7.696 ± 0.489
51	1,2,4,5-Tetramethylbenzene	5.916 ± 2.600	24.19 ± 4.405	8.317 ± 2.403	4.439 ± 0.293
52	o-Xylene	3.009 ± 1.458	14.98 ± 0.969	1.150 ± 0.210	2.757 ± 0.083
53	Styrene	+	23.90 ± 2.978	3.232 ± 1.075	2.273 ± 0.238
54	p-Xylene	+	+	+	1.350 ± 0.193
55	2-Methylnaphthalene	+	+	+	+
56	1-Methylnaphthalene	+	+	+	+
57	m-Xylene	+	-	+	+
58	Naphthalene	-	+	+	+
	Aldehydes				
59	trans-2-Heptenal	146.3 ± 52.27	182.2 ± 37.88	63.32 ± 27.77	512.3 ± 9.237
60	n-Dodecanal	10.85 ± 3.367	27.02 ± 2.698	34.00 ± 13.17	306.6 ± 1.381
61	2-Nonenal	7.093 ± 4.352	7.968 ± 2.153	-	-
62	trans-2-Decenal	6.883 ± 3.384	6.579 ± 0.213	20.63 ± 3.676	51.85 ± 0.582
63	trans,trans-2,4-Nonadienal	5.423 ± 1.750	5.095 ± 0.717	46.79 ± 9.876	43.97 ± 0.818
64	Benzaldehyde	4.888 ± 2.584	15.18 ± 4.482	28.63 ± 3.785	+
65	Octanal	2.893 ± 1.778	18.79 ± 2.964	20.86 ± 4.684	-
66	n-Decanal	2.059 ± 1.380	1.012 ± 0.200	2.310 ± 0.690	1.528 ± 0.207
67	Phenylacetaldehyde	1.288 ± 0.276	+	3.230 ± 1.539	+
68	4,5-Epoxy-(E)-2-decenal	1.023 ± 0.494	+	2.454 ± 0.802	13.88 ± 0.520
69	Salicylaldehyde	+	+	6.367 ± 1.603	1.585 ± 0.178
70	trans,trans-2,4-Heptadienal	+	+	+	+
71	Hexanal	-	50.53 ± 27.65	37.20 ± 6.896	3.221 ± 0.116
72	trans,trans-2,4-Decadienal	-	+	1.007 ± 0.351	+
	Pyrazines				
73	2-Ethylpyrazine	101.6 ± 51.58	25.37 ± 2.615	232.6 ± 47.45	542.1 ± 15.60
74	2,3-Dimethylpyrazine	1.902 ± 0.993	1.522 ± 0.097	7.433 ± 0.932	12.10 ± 0.216
75	2-Isopropyl-3-methoxypyrazine	+	+	+	+
76	2-Isobutyl-3-methoxy pyrazine	+	+	+	+
77	2-Methylpyrazine	+	+	1.746 ± 0.336	+
	Alcohols				
78	1-Octanol	11.31 ± 6.357	76.55 ± 4.184	11.03 ± 2.921	183.3 ± 1.744
79	Benzyl alcohol	7.046 ± 3.049	17.83 ± 0.979	8.074 ± 4.790	1.643 ± 0.042
80	2-Nonanol	2.438 ± 1.666	7.968 ± 0.604	15.61 ± 11.01	1.732 ± 0.213

Table 2. *Cont.*

No.	Component	Chuanxiong (ng/g, *n* = 10)	Japanese Chuanxiong (ng/g, *n* = 3)	Fuxiong (ng/g, *n* = 7)	Jinxiong (ng/g, *n* = 3)
81	1-Tetradecanol	1.046 ± 0.357	-	-	14.77 ± 0.542
82	1-Dodecanol	+	+	1.767 ± 0.770	32.34 ± 0.231
83	1-Undecanol	-	+	2.050 ± 0.936	+
84	2-Phenylethanol	-	-	-	3.252 ± 0.013
85	1-Pentanol	-	3.119 ± 0.225	-	-
86	2-Ethyl-1-hexanol	-	-	8.34 ± 8.039	3.244 ± 0.269
	Esters				
87	Ethyl acetate	10.23 ± 6.942	3.195 ± 1.161	173.3 ± 34.61	173.2 ± 8.945
88	n-Hexyl acetate	1.997 ± 0.674	3.980 ± 0.342	1.129 ± 0.457	6.266 ± 0.156
89	n-Butyl acetate	1.795 ± 0.804	+	2.662 ± 1.808	9.478 ± 0.150
90	1-Methoxy-2-propyl acetate	+	2.431 ± 0.699	+	+
91	Methyl methacrylate	+	1.669 ± 0.530	+	1.046 ± 0.087
92	Ethyl sorbate	+	1.012 ± 0.200	6.218 ± 1.155	37.61 ± 0.119
93	Methyl salicylate	+	+	3.396 ± 0.669	4.452 ± 0.257
94	gamma-Octalactone	+	+	+	+
95	gamma-Decalactone	+	+	+	+
96	gamma-Dodecalactone	+	+	+	+
97	2-Ethoxyethyl acetate	-	+	-	1.173 ± 0.319
98	Ethyl-2-methylbutyrate	-	+	-	+
99	sec-Butyl acetate	-	-	+	+
	Phenols				
100	Methyleugenol	3.275 ± 0.763	3.541 ± 1.465	6.622 ± 2.278	238.3 ± 0.411
101	Vanillin	2.017 ± 1.097	11.34 ± 4.201	3.995 ± 1.234	3.310 ± 0.239
102	Eugenol	+	4.435 ± 0.347	+	22.38 ± 0.365
103	6-Chloro-o-cresol	+	+	3.312 ± 1.158	8.473 ± 0.384
104	m-Cresol	+	+	+	1199 ± 78.04
105	p-Cresol	+	+	+	432.2 ± 9.016
106	p-Ethylphenol	+	+	+	5.326 ± 0.217
107	o-Cresol	+	+	+	7.189 ± 0.176
108	2,3-Xylenol	+	+	+	+
109	p-Ethylguaiacol	+	+	+	+
110	p-Propylphenol	+	+	+	+
111	Phenol	-	+	+	3.692 ± 0.017
112	Geosmin	-	+	+	+
113	Guaiacol	-	-	-	+
	Pyridines				
114	5-Ethyl-2-methylpyridine	2.487 ± 1.609	4.651 ± 1.421	5.920 ± 1.676	7.609 ± 0.236
115	3-Ethyl-4-methylpyridine	+	+	6.877 ± 0.885	+
116	2-n-Propylpyridine	+	+	1.075 ± 0.289	1.112 ± 0.013
	Ethers				
117	Diethyl disulfide	1.207 ± 0.789	1.271 ± 0.203	10.63 ± 3.640	56.00 ± 2.100
118	Dimethyl disulfide	+	1.432 ± 0.299	+	1.150 ± 0.463
119	Butyl cellosolve	+	+	+	3.208 ± 0.011
120	Dimethyl trisulfide	-	+	+	+
121	2,4,6-Trichloroanisole	-	-	+	+
122	2-Phenoxyethanol	-	-	-	4.546 ± 0.323
	Others				
123	5-Methyl furfural	+	+	3.012 ± 0.813	+
124	p-Dichlorobenzene	+	+	+	+
125	Caprolactam	+	+	+	+
126	Coumarin	+	+	+	+
127	Skatole	+	+	+	+
128	Benzothiazole	+	+	+	-

Data are expressed as $\bar{x} \pm s$. (mean ± standard deviation); "-" Not detected. "+" Detected, but below the limit of quantification.

However, volatile compounds were found to be varied among these herbs. Each herb has its unique volatile compounds. There were 9, 18, 19, and 23 unique volatile compounds found in CX, JCX, FX, and JX, respectively. Further comparison of the four herbs showed that there were 103 common volatile compounds in CX and JCX. Only three volatile compounds, namely eucalyptol (18), m-xylene (57), and 1-tetradecanol (81), were found in CX but not found in JCX, while 12 volatile compounds existed in JCX but were not found in CX. Meanwhile, 102 volatile compounds were simultaneously detected in CX and FX. However, only 4 volatile compounds, i.e., eucalyptol (18), ethylic acid (20), 2-nonenal (61), and 1-tetradecanol (81), were detected in CX, and not found in FX, whilst 14 compounds were found in FX, but not detected in CX. Similarly, there were 99 common volatile compounds in CX and JX. However, 5 compounds were found only in CX, i.e., eucalyptol (18), ethylic acid (20), 2-nonenal (61), octanal (65), and benzothiazole (128), but not in JX, while 21 compounds that existed in JX were not found in CX. In addition, JCX contained 109 of the same compounds as FX and 107 compounds in common with JX. FX and JX contained 110 common volatile compounds. These results indicated volatile compounds varied in the four herbs. CX contained more similar volatile compounds with JCX, followed by FX, and then JX. Meanwhile, these unique volatile compounds should be used as a chemical marker to differentiate these from each other.

3.6. Comparing the Contents of Volatile Compounds in the Four Herbs

In order to compare the contents of volatile compounds in these herbs, the concentrations of the specific volatile compounds were calculated on the basis of the regression equation of the 154 chemical standards in the developed database (Table S1). The contents of the 128 compounds were found to be significantly different in the volatile gas of the four herbs (Table 2). The total contents of detected compounds in the volatile gas of CX, JCX, FX, and JX were 4.80 μg/g (n = 10), 7.12 μg/g (n = 3), 7.67 μg/g (n = 7), and 12.0 μg/g (n = 3), respectively. The ratios of the contents of 97 types of common volatile compounds to the total detected volatile compounds in CX, JCX, FX, and JX were 94.3%, 95.0%, 99.2%, and 92.6%, respectively. The results indicated the contents of volatile compounds were somewhat different among the four herbs.

The difference of the contents of individual volatile compounds among these herbs were further compared. The variation was found to be from 0.83% to 50.00% for individual compounds in the 128 detected volatile compounds of the four herbs. If the similar concentration compound was defined as a compound with a concentration variation of less than 10% in these four herbs, only phenylacetic acid (25) and senkyunolide A (2) were similar in concentration compounds, with a variation of 0.83% and 9.98%, respectively. Further analyzing the compounds between these two herbs found the quantities of similar concentration compounds were 27 for CX and JCX, 17 for CX and FX, and 13 for CX and JX. Similarly, there were 20 similar concentration compounds in JCX and FX, 22 in JCX and JX, and 26 in FX and JX. The results also demonstrated that the contents of volatile compounds were different in the four herbs. CX was much closer to JCX, followed by FX, and then JX.

3.7. Comparing the Main Volatile Compounds in the Four Herbs

The sum of 128 compounds were quantified in the volatile gas of the four herbs. The contents of each volatile compound were significantly different among these herbs (Table 2). The main volatile compounds comprised only a few compounds. The contents of the top ten compounds were 87.9%, 82.6%, 82.9%, and 76.1% in the total detected volatile compounds in CX, JCX, FX, and JX, respectively (Table 3). In particular, the ratios of the top five compounds were 69.9%, 67.5%, 66.8%, and 48.7% in the total detected volatile compounds in CX, JCX, FX, and JX, respectively.

Table 3. The top ten compounds in the detected volatile compositions of the four herbs.

No.	Chuanxiong		Japanese Chuanxiong		Fuxiong		Jinxiong	
	Compound	**Ratio (%)**	**Compound**	**Ratio (%)**	**Compound**	**Ratio (%)**	**Compound**	**Ratio (%)**
1	Ligustilide	22.5	2-Undecanone	22.0	Ligustilide	22.4	Ligustilide	12.8
2	Senkyunolide A	17.9	alpha-Pinene	20.3	Senkyunolide A	13.3	m-Cresol	10.0
3	alpha-Pinene	16.6	Ligustilide	11.2	beta-Pinene	13.2	Limonene	9.2
4	2-Undecanone	8.1	Senkyunolide A	7.4	alpha-Pinene	10.0	Senkyunolide A	8.4
5	Ethylic acid	5.2	Butyric acid	4.0	2-Undecanone	7.0	3-Butylidenephthalide	8.2
6	Toluene	5.1	Linalool	3.9	3-Butylidenephthalide	4.1	alpha-Pinene	7.8
7	Limonene	3.7	beta-Pinene	3.5	Limonene	3.8	Acetoin	7.1
8	Butyric acid	3.1	Ethylic acid	3.5	Linalool	3.3	2-Ethylpyrazine	4.5
9	trans-2-Heptenal	3.0	Toluene	3.4	2-Ethylpyrazine	3.1	trans-2-Heptenal	4.3
10	Linalool	2.4	Caproic acid	3.1	Butyric acid	2.5	p-Cresol	3.6

The specific main compounds were also different in the volatile gas of the four herbs. Although ligustilide and senkyunolide A were the well-known main compounds in these herbs, ligustilide was the first major compound in CX (22.5%), FX (22.4%), and JX (12.8%), whilst it was the third major compound in JCX (5.84%). Senkyunolide A was the second major compound in CX (17.9%) and FX (13.2%), and it was the fourth major compound in JCX (9.79%) and JX (8.41%). It was worth noticing that the first and second major compounds in JCX were 2-undecanone (22.9%) and alpha-pinene (21.1%), respectively.

3.8. Comparing the Chemical Classes of Volatile Compounds in the Four Herbs

The chemical classes of these volatile compounds were further compared (Table 4). All four herbs contained the same classes of chemical compounds, i.e., phthalides, terpenes, organic acids, ketones, aromatics, aldehydes, pyrazines, alcohols, esters, phenols, pyridines, ethers, and others. Phthalides and terpenoids were the main chemical classes, with ratios of 69.0%, 49.7%, 72.8%, and 52.5% in the detected volatile compounds in CX, JCX, FX, and JX, respectively. The results indicated that the volatile chemical compositions had a resemblance among these four herbs.

Table 4. The quantity and ratio of volatile compounds detected in specific chemical classes in the four herbs.

No.	Chemical Classes	Chuanxiong		Japanese Chuanxiong		Fuxiong		Jinxiong	
		Compound Number	**Content Percentage**	**Compound Number**	**Content Percentage**	**Compound Number**	**Content Percentage**	**Compound Number**	**Content Percentage**
1	Phthalides	4	42.17	4	16.51	4	40.58	4	30.07
2	Terpenoids	15	24.84	13	33.14	14	32.24	14	22.44
3	Organic acids	12	10.96	14	14.33	14	7.03	14	2.16
4	Ketones	11	8.71	11	23.98	11	7.89	13	9.96
5	Aromatics	10	6.27	10	4.86	11	1.86	11	2.26
6	Aldehydes	12	3.90	14	4.73	14	3.62	13	7.84
7	Pyrazines	5	2.09	5	0.40	5	3.18	4	4.64
8	Alcohols	5	0.46	6	1.40	5	0.50	7	2.00
9	Esters	10	0.32	12	0.22	11	2.50	13	1.96
10	Phenols	11	0.14	13	0.30	13	0.21	14	16.04
11	Pyridines	3	0.06	3	0.08	3	0.19	3	0.08
12	Ethers	3	0.04	4	0.04	5	0.15	6	0.54
13	Others	6	0.03	6	0.01	6	0.05	4	0.003
	Total	106	100	115	100	116	100	120	100

However, there were differences in the types and contents of volatile compounds in specific chemical classes. The top three chemical classes of high ratios were different in the four herbs, i.e., phthalides (42.2%), terpenoids (24.8%), and organic acids (11.0%) in CX; terpenoids (33.1%), ketones (24.0%), and phthalides (16.5%) in JCX; phthalides (40.6%), terpenoids (32.2%), and ketones (7.9%) in FX; and phthalides (30.1%), terpenoids (22.4%), and phenols (16.0%) in JX.

3.9. Statistical Analysis of Volatile Compounds in the Four Herbs

In order to holistically compare the volatile composition among the four herbs, OPLS-DA was applied to analyze the volatile compounds among the 23 samples in the four herbs.

The values of R2X, R2Y, and Q2 of OPLS-DA were 0.797, 0.985, and 0.972, respectively, which implied that the adaptability and accuracy of the developed model were good. The samples of CX, JCX, FX, and JX were located in different confined areas (Figure 2). This demonstrated the sameness for samples in specific herbs and the difference for samples among different herbs. Moreover, the HCA results showed that CX and JCX were clustered into one group, whilst FX and JX were clustered into another group (Figure 3). The results implied the volatile odors in the four herbs were different, while CX and JCX were more similar, and FX and JX were more similar.

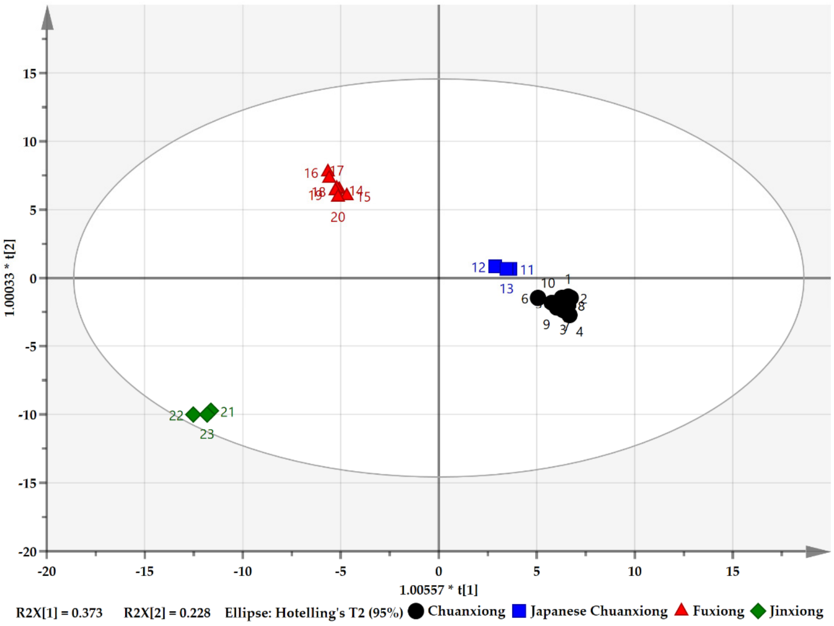


Figure 2. The plot of orthogonal partial least–squares discrimination analysis (OPLS-DA). 1–10: Chuanxiong (*Ligusticum chuanxiong*); 11–13: Japanese Chuanxiong (*Cnidium officinale*); 14–20: Fuxiong (*Ligusticum sinense* 'Fuxiong'); and 21–23: Jinxiong (*Ligusticum sinense* 'Jinxiong').

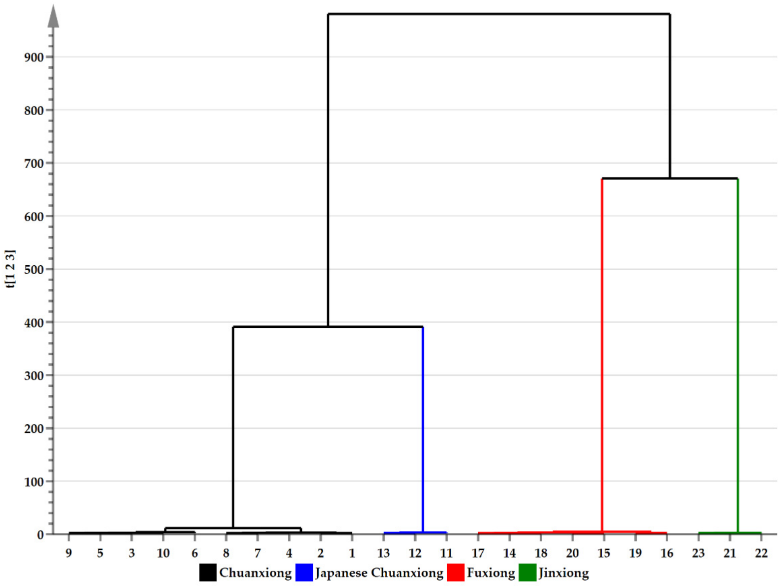


Figure 3. The hierarchical clustering analysis (HCA) of the four herbs. 1–10: Chuanxiong (*Ligusticum chuanxiong*); 11–13: Japanese Chuanxiong (*Cnidium officinale*); 14–20: Fuxiong (*Ligusticum sinense* 'Fuxiong'); and 21–23: Jinxiong (*Ligusticum sinense* 'Jinxiong').

4. Discussion

4.1. The Developed Method Can Rapidly Detect the Natural and Real Compounds in Volatile Gas and Is a Novel Method for the Analysis of Aromatic Herbs

Aromatic herbs contain volatile matters. The aromatic odor is one of the main indicators for the genuine authentication and the quality assessment of aromatic herbs [8]. Extraction of volatile compounds is necessary for qualitative and quantitative analyses of aromatic herbs. However, the traditional extraction methods of the volatile oil are conducted under heating conditions, e.g., steam distillation and reflux extraction, which results in the oxidation and/or decomposition of volatile compounds [14]. Hence, natural and real volatile compounds are not easily obtained. In the present study, the HS-SPME technique is used to produce the volatile gas from herb powders under a relatively low heating temperature, then the volatile gas is directly absorbed into the GC instrument to analyze the natural and real volatile compounds in aromatic herbs. Meanwhile, the triple quadrupole–mass spectrometry (QQQ-MS) is combined with HS-SPME-GC, which improves the accuracy, reproducibility, and realness of analysis data for volatile compounds. The HS-SPME-GC-QQQ-MS method is also rapid, with high sensitivity. In this study, this method is employed to detect the volatile compounds in the related herbs CX, JCX, FX, and JX. The results are real, accurate, and comparable. The developed method may offer extended uses for the analysis of volatile compounds in other aromatic herbs.

4.2. The Volatile Compositions Are Generally Similar, but the Amounts of Major Bioactive Compounds Are Obviously Different, Indicating That the Four Herbs Should Be Differentiated for Their Medical Uses

In the plant taxonomy, the relationship of CX, JCX, FX, and JX are very close [6,19–21]. Their plant origins are likely derived from the same plant species, *Ligusticum sinense* Oliv. [4,22]. This results in a similar morphology, aromatic odor, and herb name. These herbs are cultivated in China, Japan, Korea, etc. CX and JCX are officially used in traditional medicine and recorded in the Chinese Pharmacopoeia or Japanese Pharmacopeia [1,23]. They play an almost identical medical role in traditional medicine in China or Japan in prescriptions or proprietary medicines, e.g., *Siwu Decoction* [24]. FX and JX are folk medicines and have not yet been recorded in Chinese Pharmacopoeia. They are usually used in healthcare functions. However, these four herbs are easily confused and sometimes have mixed uses.

Traditionally, aromatic odor is the common main indicator to evaluate the authenticity and quality of these herbs. However, this is not a scientific approach. In this study, 23 samples of the four herbs are collected from different cultivated areas in China. A total of 128 compounds are qualitatively and quantitatively analyzed in the volatile gas of these herbs by HP-SPME-GC-QQQ-MS. The classes and contents of volatile compounds are identified, quantitatively compared, and statistically analyzed. The quantities of chemical classes volatile compounds are the same in the four herbs, while the classes of volatile compounds are also similar. There are 97 common compounds among the 128 detected compounds in the four herbs. The ratio of common compounds is 75.8% in these herbs. The GC-MS chromatograms of the volatile gas from the four herbs are generally similar. It is concluded that the volatile compositions are generally similar in the four related herbs. CX and JCX are more similar, followed by CX and FX, and lastly, CX and JX.

However, the contents of the main volatile compounds are found to be different. The total contents of detected compounds in the volatile gas are significantly different, with various mean contents of 4.80 μg/g for CX, 7.12 μg/g for JCX, 7.67 μg/g for JX, and 12.0 μg/g for JX. The classes and contents of the main volatile compounds are also different in the volatile gas of these herbs. For example, ligustilide, senkyunolide A, alpha-pinene, 2-undecanone, and ethylic acid are the top five high-content compounds in CX. They are bioactive compounds possessing different efficacy, such as vasodilation, anti-platelet aggregation, anti-coagulation, anti-tumor, and anti-inflammatory properties. However, ethylic acid is not found in the volatile gas of FX and JX. The ratios of the other four compounds are obviously different in the four herbs. OPLS-DA of whole GC-MS chromatograms shows that the four herbs are located in different confined areas, even though CX and JCX are closer. HCA shows the same results as OPLS-DA. These differences imply that the medical efficacy of the four herbs may be not identical for the four herbs. Therefore, these herbs should still be differentiated in medical uses.

In Chinese medicine theory, CX is used to activate blood circulation and resolve blood stasis. Although JCX plays an almost identical role in prescriptions or proprietary medicines, for traditional usage, they are not interchangeable. FX and JX cannot yet replace CX as a formal drug. However, the bioactivity and clinical efficacy should be further compared among these herbs to confirm the sameness and differences.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/pr11010196/s1, Table S1: Retention time, mass spectrometry detection parameters, and regression equation of standards; Table S2: N-alkane reference and test data.

Author Contributions: Conceptualization, F.L., K.C. and G.L.; formal analysis, S.H., J.C., H.Y. and X.B.; investigation, S.H., J.C., W.L., X.L. and X.B.; methodology, S.H., X.B. and G.L.; resources, S.H., J.C., W.L., S.S., X.L. and F.L.; software, S.H., J.C. and W.L.; supervision, F.L. and G.L.; visualization, S.H., J.C., W.L. and G.L.; writing—original draft, S.H., J.C. and W.L.; writing—review and editing, F.L., R.C., K.C. and G.L. All authors have read and agreed to the published version of the manuscript.

Funding: The research work was funded by the National Natural Science Foundation of China (81773896) and Sichuan Science and Technology Programs (2017SZ0156).

Data Availability Statement: Not applicable.

Acknowledgments: We are thankful for Lan Cao, Shouwen Zhang, and Weiwei Gong at Jiangxi University of Traditional Chinese Medicine for collecting some of the herbal samples in Jiangxi province of China, and Jianfeng Xu for collecting herbs in Yunnan province of China.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Pharmacopoeia Committee of Japanese. *Japanese Pharmacopoeia*; Ministry of Health and Welfare Press: Tokyo, Japan, 2021; Volume 1, p. 1229.
2. Kimura, K.; Hata, K.; Kozawa, M. Pharmacognostical studies on Umbelliferous plants XVII. *Syoyakugaku Zasshi* **1961**, *15*, 5–13.
3. Suk, K.-T.; Nitta, A.Y.A.; Konoshima, M. Studies on origin of Japanese Chuanxiong. VI. *Yakugaku Zasshi* **1974**, *94*, 1270–1273. [CrossRef] [PubMed]
4. Lee, S.-H.; Choi, H.-W.; Sung, J.-S.; Bang, J.-W. Inter-genomic relationships among three medicinal herbs: Cnidium officinale, Ligusticum chuanxiong and Angelica polymorpha. *Genes Genom.* **2010**, *32*, 95–101. [CrossRef]
5. Jin, W.; Cui, J.; Liu, Y. Study on the cultivation technique of Dongchuanxiong. *J. Tradit. Chin. Med.* **2001**, *6*, 26–27.
6. Pu, F.D.; Watson, M. *Apiaceae. Ligusticum L. Flora of China*; Scientific Press: Beijing, China, 2005; Volume 14, pp. 140–150.
7. Zhang, H.; Fang, S.; Wu, L. Studies on the origin of the traditional Chinese drug "Jinxiong". *Acta Pharmacol. Sin.* **1990**, *28*, 447–482.
8. Gündeşli, M.A.; Kafkas, N.E.; Okatan, V.; Usanmaz, S. Identification and characterisation of volatile compounds determined by Hs/Gcms technıque in pulp of 'Abbas' Fig (*Ficus carica* L.) variety. *Pak. J. Agri. Sci.* **2020**, *57*, 623–629. [CrossRef]
9. Wan, D.; Peng, C.; Zhang, H. *Records of Genuine Medicinal Materials in Sichuan*; Sichuan Science and Technology Press: Chengdu, China, 2005; pp. 4–12.
10. Kafkas, N.E.; Gündeşli, M.A. *Fig Flavor. Advances in Fig Research and Sustainable Production*; CABI: Wallingford, UK, 2022; pp. 364–386. [CrossRef]
11. Yang, C.; Wang, C.; Wang, M.; Qin, X.; Hao, G.; Kang, M.; Hu, X.; Cheng, Y.; Shen, J. A novel deodorization method of edible oil by using ethanol steam at low temperature. *J. Food Sci.* **2021**, *86*, 394–403. [CrossRef] [PubMed]
12. Dal Prá, V.; Dolwitsch, C.B.; da Silveira, G.D.; Porte, L.; Frizzo, C.; Tres, M.V.; Mossi, V.; Mazutti, M.A.; do Nascimento, P.C.; Bohrer, D.; et al. Supercritical CO_2 extraction, chemical characterisation and antioxidant potential of Brassica oleracea var capitata against HO, O_2^- and ROO. *Food Chem.* **2013**, *141*, 3954–3959. [CrossRef] [PubMed]
13. Zahiri, E.; Khandaghi, J.; Farajzadeh, M.A.; Afshar Mogaddam, M.R. Combination of dispersive solid phase extraction with solidification organic drop-dispersive liquid-liquid microextraction based on deep eutectic solvent for extraction of organophosphorous pesticides from edible oil samples. *J. Chromatogr. A* **2020**, *1627*, 461390. [CrossRef] [PubMed]
14. Cui, Y. The Study on Variations of Chemical Componets and Antioxidant Activity of Red Wine During Decanting Process. Ph.D. Thesis, Shenyang Pharmaceutical University, Shenyang, China, 2011.
15. Zhang, C.; Qi, M.; Shao, Q.; Zhou, S.; Fu, R. Analysis of the volatile compounds in Ligusticum chuanxiong Hort. using HS-SPME-GC-MS. *J. Pharm. Biomed. Anal.* **2007**, *44*, 464–470. [CrossRef] [PubMed]

16. Deng, H.; He, R.; Long, M.; Li, Y.; Zheng, Y.; Lin, L.; Liang, D.; Zhang, X.; Liao, M.; Lv, X.; et al. Comparison of the fruit volatile profiles of five muscadine grape cultivars (Vitis rotundifolia Michx.) using HS-SPME-GC/MS combined with multivariate statistical analysis. *Front. Plant Sci.* **2021**, *12*, 728891. [CrossRef] [PubMed]
17. Peng, Y.; Bishop, K.S.; Zhang, J.; Chen, D.; Quek, S.Y. Characterization of phenolic compounds and aroma active compounds in feijoa juice from four New Zealand grown cultivars by LC-MS and HS-SPME-GC-O-MS. *Food Res. Int.* **2020**, *129*, 108873. [CrossRef] [PubMed]
18. Li, A.; Duan, S.; Dang, Y.; Zhang, X.; Xia, K.; Liu, S.; Han, X.; Wen, J.; Li, Z.; Wang, X.; et al. Origin identification of Chinese Maca using electronic nose coupled with GC-MS. *Sci. Rep.* **2019**, *9*, 12216. [CrossRef] [PubMed]
19. Wang, P. Pollen morphology and ITS relationship of Ligusticum sinense cv. Chuanxiong, L. sinense cv. Fuxiong and L. sinense cv. *Acta Bot. Yunnanica* **1990**, *2*, 173–178, 238–240.
20. Zhou, J.; Gao, Y.; Wei, J.; Liu, Z.; Downie, S.R. Molecular phylogenetics of Ligusticum (Apiaceae) based on nrDNA ITS sequences: Rampant polyphyly, placement of the Chinese endemic species, and a much-reduced circumscription of the genus. *Int. J. Plant Sci.* **2020**, *181*, 306–323. [CrossRef]
21. Ren, T.; Xie, D.; Peng, C.; Gui, L.; Price, M.; Zhou, S.; He, X. Molecular evolution and phylogenetic relationships of Ligusticum (Apiaceae) inferred from the whole plastome sequences. *BMC Ecol. Evol.* **2022**, *22*, 55. [CrossRef] [PubMed]
22. Yuan, C.; Sha, X.; Xiong, M.; Zhong, W.; Wei, Y.; Li, M.; Tao, S.; Mou, F.; Peng, F.; Zhang, C. Uncovering dynamic evolution in the plastid genome of seven Ligusticum species provides insights into species discrimination and phylogenetic implications. *Sci. Rep.* **2021**, *11*, 988. [CrossRef] [PubMed]
23. Pharmacopoeia Committee of Chinese. *Pharmacopoeia of the People's Republic of China*; Medical Science Press: Beijing, China, 2020; Volume 1, p. 42.
24. He, Y. Literature research and clinical application of Japanese Siwu Decoction. *Foreign Med. Sci. Tradit. Chin. Med.* **1994**, *5*, 1–4.