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Chemical Characterization and Quality Evaluation of *Forsythia suspensa* Flowers Based on HS-GC-MS, UPLC-QTOF-MS and HPLC-DAD Analysis

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Abstract

Forsythia suspensa is a medicinal and edible shrub, whose fruit is a commonly used herbal medicine. *Forsythia suspensa* flowers (FFs) exhibit significant edible and medicinal potential, but research on the chemical composition and quality evaluation remain limited. In this study, HS-GC-MS, UPLC-QTOF-MS and HPLC-DAD were combined for the chemical characterization and quality evaluation of FFs. HS-GC-MS was used to characterize volatile components and build fingerprints of FFs. UPLC-QTOF-MS was applied for qualitative analysis of non-volatile constituents, and the HPLC fingerprint was established to assess the consistency of FF samples. A validated HPLC-DAD method was adopted for determination of seven non-volatile components (chlorogenic acid, rutin, forsythoside A, (+)-pinoresinol-4-O-β-D-glucopyranoside, phillyrin, quercetin and phillygenin) in FF samples. Forty-four volatile and thirty-two non-volatile compounds were characterized. HS-GC-MS and HPLC fingerprints of FF were established, and fifteen volatile and seven non-volatile constituents were identified as common peaks. The established GC-MS and HPLC fingerprint method had good specificity and could be used for the quality evaluation of FF. Chemometric based on quantitative results showed significant differences in non-volatile constituents in FF samples from different producing regions. This work provided a reliable method and useful information for the chemical characterization and quality evaluation of FF. Further research will focus on biological activities of FF and its active components, laying a foundation for its application in pharmaceutical and food industries.

Keywords: *Forsythia suspensa* flowers; quality evaluation; chemical characterization; HS-GC-MS; UPLC-QTOF-MS

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1. Introduction

Scented tea, a traditional herbal tea renowned for its health benefits, has been widely consumed in China for a long time. Crafted from the flowers of various plants, such as rose, jasmine and plum, it boasts a distinctive aroma and flavor, along with unique

therapeutic properties [1]. *Forsythia suspensa* (Thunb.) Vahl is an ornamental shrub widely distributed in East Asia and Europe. The fruit of *Forsythia suspensa* is a well-known traditional Chinese medicine, commonly used to treat pyrexia, inflammation, gonorrhea, carbuncle and erysipelas in clinical therapies [2]. In addition to the fruit, the leaves and flowers of *Forsythia suspensa* also have considerable edible and medicinal value. *Forsythia suspensa* flower (FF) is often crafted into health-promoting scented tea, which has been a tradition in northern China's folk culture [3,4].

Currently, phytochemical studies showed FF was rich in bioactive compounds, including polyphenols, flavonoids, lignans and triterpene acids [4,5]. The types of bioactive components contained in FF are similar to those in *Forsythia suspensa* fruits and leaves, which have strong health-promoting properties. Numerous studies have confirmed that FF contains forsythoside A, rutin, (+)-pinoresinol-4-O-β-D-glucopyranoside, along with other bioactive constituents [6]. Modern pharmacological research found that FF and bioactive constituents present in FF had multiple pharmacological activities, such as anti-inflammatory, anti-oxidation, anti-diabetes, anti-hyperlipidemia and neuroprotection [7,8]. Therefore, FF is a good potential plant resource and has great development value. The primary medicinal part of *Forsythia suspensa* is its fruit, prompting farmers to remove large quantities of flowers to improve the fruit setting rates. This practice leads to the disposal of a substantial amount of FF, resulting in a considerable waste of valuable resources. The major producing regions of *Forsythia suspensa* in China include Shanxi, Hebei, Henan and Shandong provinces, and thousands of tons of FF are discarded annually during flower-thinning, causing substantial waste of this valuable plant resource. Additionally, most recent studies focused on chemical characterization and quality control of *Forsythia suspensa* fruits and leaves, but there has been no study and discussion on comprehensive chemical characterization and quality evaluation of FFs. Such a study is thus urgently needed for the comprehensive chemical characterization of FF and development of a quality evaluation method for FF.

In the field of plant chemical composition analysis and quality control, several advanced chromatographic techniques are commonly employed, such as high-performance liquid chromatography (HPLC), gas chromatography–mass spectrometry (GC-MS) and liquid chromatography–mass spectrometry (LC-MS) [9]. GC-MS and LC-MS have attained considerable prominence in chemical composition analysis, owing to their outstanding analytical versatility, excellent separation efficiency, high analytical throughput, and remarkable capacity for comprehensive metabolite profiling [10]. Headspace (HS) in sealed vials is a non-destructive analytical method targeting the volatile aroma components of samples, which features simple operation, high efficiency, low cost and favorable safety performance [11]. The chemical fingerprint can serve as a powerful analytical approach for characterizing the chemical profiles of complex systems. This approach has been extensively applied in quality control of herbal medicine and comprehensive phytochemical profiling of plant materials [12]. Therefore, the combination of chromatographic techniques (HS-GC-MS, LC-MS, HPLC) and chemical fingerprint analysis not only can fully characterize the chemical constituents of FF, but can also offer strong support for constituent identification and quality evaluation of FF.

In this present study, multiple chromatographic analyses were employed for the comprehensive chemical characterization and quality evaluation of FF. An HS-GC-MS method was applied to identify the volatile constituents of FF, and a GC-MS-based fingerprint was established to evaluate the similarities and differences in volatile components among different FF batches. Meanwhile, a UPLC-QTOF-MS technique was used for the qualitative analysis of non-volatile constituents in FF. Furthermore, an HPLC-based fingerprint was established to assess the batch-to-batch consistency of FF samples from different producing

areas. In addition, a validated HPLC-DAD method was adopted for the simultaneous quantitative analysis of seven non-volatile components in FF samples. This study could provide reliable technical support for the chemical profiling and quality control of FF, as well as offer a theoretical basis for the development and application of FF in the pharmaceutical and food industries.

2. Materials and Methods

2.1. Plant Materials

A total of twenty batches of FF samples were collected from four main production areas in China, including Shandong (SD) province (S1–S3), Henan (HN) province (S4–S7), Shanxi (SX) province (S8–S16) and Hebei (HB) province (S17–S20). The information of FF samples is displayed in Supplementary Table S1. The FF samples were collected in April 2024 at the anthesis stage (fully open flowers, prior to petal abscission) within a 10-day harvest window to minimize compositional variation caused by the flower developmental stage. After collection, the fresh FF were evenly spread in a well-ventilated dark room at 20–25 °C with relative humidity 45–60% for shade-drying. The samples were authenticated by Prof. Long Guo, and the voucher specimens were displayed in Traditional Chinese Medicine Processing Technology Innovation Center of Hebei Province, Hebei University of Chinese Medicine.

2.2. Chemicals and Reagents

Reference compounds, chlorogenic acid, rutin, forsythoside A, (+)-pinoresinol-4-O-β-D-glucopyranoside, phillyrin, quercetin and phillygenin were provided by Chengdu Pusi Biotechnology Co., Ltd. (Chengdu, China). Acetonitrile, methanol and formic acid (HPLC grade) were purchased from Fisher Scientific (Fair Lawn, NJ, USA). Purified water was prepared by a Synergy water purification system (Millipore, Billerica, MA, USA). All other analytical reagents were purchased from Tianjin Yongda Chemical Reagent Co., Ltd. (Tianjin, China).

2.3. Sample Preparation

2.3.1. Volatile Oil Extraction

200 g of FF was accurately weighed and transferred into a volatile oil extraction apparatus. A total of 3000 mL of distilled water was added, and then macerated for 30 min. Subsequently, the mixture was heated and kept at a gentle boil for 5 h. After the volatile oil was collected, the sample was dehydrated with anhydrous sodium sulfate to afford a pale-yellow aromatic volatile oil. The volatile oil sample was diluted 20 times by n-hexane before HS-GC-MS analysis.

2.3.2. Preparation of Sample Solution

FF samples were pulverized and ground into powder and then passed through a 40-mesh sieve. A 0.20 g sample of the powder was accurately weighed and subjected to ultrasonic extraction with 20 mL of 80% (v/v) methanol for 40 min at 250 W and 100 kHz. After cooling to room temperature, the extraction was adjusted to the original weight with 80% (v/v) methanol. The extraction was then centrifuged at 13000 r/min for 10 min, and the supernatant was filtered through a 0.22 μm microporous membrane prior to HPLC-DAD analysis. Furthermore, the resultant filtrate was diluted 5-fold prior to UPLC-QTOF-MS analysis.

2.3.3. Preparation of Standard Solution

The stock standard solution of chlorogenic acid, rutin, forsythoside A, (+)-pinoresinol-4-O-β-D-glucopyranoside, phillyrin, quercetin and phillygenin were prepared by accurately weighing each reference substance and dissolving them in 80% (*v*/*v*) methanol with the concentrations of 627.50, 7014.00, 7266.00, 7294.00, 3570.00, 224.00 and 3430.00 μg/mL, respectively. To prepare a series of working standard solutions, the above stock standard solution was gradually diluted with 80% (*v*/*v*) methanol to a series of appropriate concentrations. All solutions were stored at 4 °C before use.

2.4. HS-GC-MS Analysis

The HS-GC-MS analysis was performed on an Agilent 7890B gas chromatography with an Agilent 7697A headspace sampler and an Agilent 5977B mass detector (Agilent Technologies, Santa Clara, CA, USA). A 20 μL sample of diluted volatile oil was precisely measured and placed in a 20 mL headspace vial. The headspace conditions were set as strip heating box 120 °C, quantitative ring 130 °C, transmission line 140 °C, and equilibration time 20 min. Analytes were separated on an Agilent HP-5 MS column (5% phenylmethyl polysiloxane, 0.25 mm × 30 m, 0.25 μm). The column temperature program was initiated at 60 °C and held for 3 min. Subsequently, the temperature was raised to 120 °C at a rate of 3 °C/min and held for 3 min, followed by further heating to 240 °C at 6 °C/min with a final hold time of 1 min. The injector temperature was maintained at 250 °C. High-purity helium (99.999%) was employed as the carrier gas at a constant flow rate of 1.1 mL/min with a split ratio of 20:1. The mass spectrometer was operated in electron impact (EI) ionization mode at 70 eV, and the mass scanning range was set from *m*/*z* 30 to 400. A solvent delay time of 3 min was applied. The ion source temperature and quadrupole temperature were set at 230 °C and 150 °C, respectively.

2.5. UPLC-QTOF-MS Analysis

UPLC-QTOF-MS analysis was conducted on an Agilent 1290 UPLC system coupled with an Agilent ZORBAX Eclipse Plus C18 column (4.6 mm × 50 mm, 1.8 μm). The mobile phase was acetonitrile (A) and 0.1% aqueous formic acid (B). The conditions of gradient elution were as follows: 0–5 min, 10–15% A; 5–25 min, 15–18% A; 25–30 min, 18–30% A; 30–35 min, 30–45% A; 35–40 min, 45–100% A. The column temperature was 30 °C, the flow rate was 0.3 mL/min, and the injection volume was 1 μL.

Mass spectrometry was performed on an Agilent 6545 QTOF mass spectrometer with an ESI source. Mass conditions were as follows: ion source was positive ion scanning mode, mass scanning range (*m*/*z*) was 120–1000 Da, capillary voltage was 3500 V, nebulizer pressure was 35 psi, fragmentor voltage was 135 V, dry gas (N_2) temperature was 320 °C, dry gas flow rate was 10 L/min, sheath gas (N_2) temperature was 350 °C, sheath flow was 11 L/min, and collision energy was 40 eV. Data acquisition was performed with MassHunter Workstation (Agilent Technologies, Santa Clara, CA, USA).

2.6. HPLC-DAD Analysis

HPLC-DAD analysis was conducted on an Agilent 1260 HPLC system coupled with an Agilent ZORBAX Eclipse Plus C18 column (4.6 mm × 50 mm, 1.8 μm). The gradient elution conditions were same as UPLC-Q/TOF-MS analysis. The injection volume was set at 5 μL, and the detected wavelength was set at 280 nm. The column temperature was 30 °C, and the flow rate was 0.3 mL/min.

2.7. Data Processing and Statistical Analysis

The volatile components were identified by the NIST 17 mass spectrometry database. The qualitative analysis of non-volatile components was performed using Qualitative Analysis 10.0 software. The accurate molecular weight and tandem mass spectral fragments were matched with database data (MassBank, PubChem) and the relevant literature. Compound structures were tentatively identified by matching their fragmentation patterns with database spectra and published literature. The similarity evaluation was carried out by using the similarity evaluation system of chromatographic fingerprints of Traditional Chinese Medicine (2012 edition). Hierarchical clustering analysis (HCA) was performed in SPSS 18.0 (SPSS Inc, Chicago, IL, USA). After Z-score standardization, HCA was conducted with square Euclidean distance and inter-groups linkage. Principal component analysis (PCA) was carried out in SIMCA 14.1 (Umetric, Umeå, Sweden), and data were preprocessed by unit variance (UV) scaling before PCA modeling. One-way ANOVA with Tukey's test was used to assess significant differences among the samples, and $p < 0.05$ was considered to indicate statistical significance. For quantitative analysis, each sample were analyzed in triplicate as technical replicates, and quantitative data were presented as mean ± standard deviation (SD).

3. Results and Discussion

3.1. Chemical Characterization of Volatile Components in FF by HS-GC-MS

In order to comprehensive characterize the volatile components of FF, an HS-GC-MS method was firstly applied to analyze the volatile oil extraction from FF samples. The total ion chromatogram of FF by HS-GC-MS is displayed in Figure 1. The volatile constituents in FF were tentatively identified by comparing the mass spectra with NIST 17 library, and a total of forty-four volatile compounds were tentatively identified including 12 aldehydes, 11 alkanes, 8 terpenoid, 4 ketones, 4 esters, 2 organic acids, 1 alcohol, 1 alkene and 1 phenol [5,13,14]. The retention time, molecular formula, molecular weight, and classification of volatile constituents identified in FF are shown in Table 1. The results (Supplementary Table S2) showed that tetradecanal (31) was the most abundant volatile compound in FF samples with a relative content of 16.44% ± 6.94%. Undecanal (17) and dodecanal (20) also existed in relatively high contents in FF samples of 12.91% ± 6.50% and 13.15% ± 5.02%, respectively. The identified results indicated that aldehydes were the most abundant components in FF, followed by alkanes and terpenoids. The types of volatile constituents in FF were found to be similar to those in *Forsythia suspensa* fruits [14].

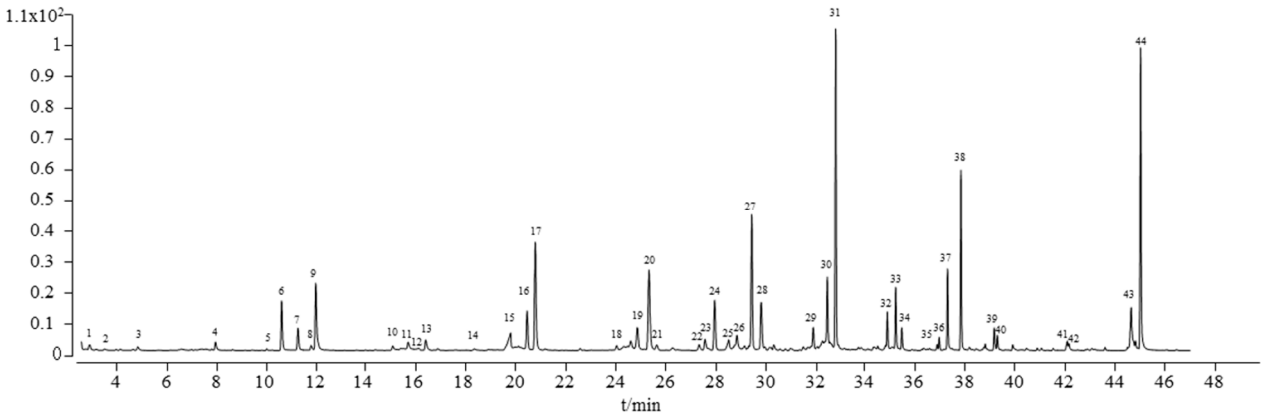


Figure 1. The total ion chromatogram of volatile components of FF by HS-GC-MS. The peak numbers are consistent with the compound numbers presented in Table 1.

Table 1. The information of volatile components identified in FF samples by HS-GC-MS.

NO.	RT (min)	Compounds	Molecular Formula	Molecular Weight	Classification
1	2.92	Hexanal	$C_6H_{12}O$	100.1	Aldehydes
2	3.52	Furfural	$C_5H_4O_2$	96.0	Aldehydes
3	4.86	Heptanal	$C_7H_{14}O$	114.1	Aldehydes
4	7.97	Octanal	$C_8H_{16}O$	128.2	Aldehydes
5	10.00	5,7-Dimethylundecane	$C_{13}H_{28}$	184.2	Alkanes
6	10.60	Linalool oxide	$C_{10}H_{18}O_2$	170.2	Alcohols
7	11.25	Furan linalool oxide	$C_{10}H_{18}O_2$	170.2	Terpenoids
8	11.78	Linalool	$C_{10}H_{18}O$	154.2	Terpenoids
9	11.95	Nonanal	$C_9H_{18}O$	142.2	Aldehydes
10	15.05	Terpinen-4-ol	$C_{10}H_{18}O$	154.2	Terpenoids
11	15.66	α-Terpineol	$C_{10}H_{18}O$	154.2	Terpenoids
12	16.09	Dodecane	$C_{12}H_{26}$	170.3	Alkanes
13	16.37	Decanal	$C_{10}H_{20}O$	156.2	Aldehydes
14	18.32	Perilla ketone	$C_{10}H_{14}O_2$	166.2	Ketones
15	19.78	Nonanoic acid	$C_9H_{18}O_2$	158.2	Fatty acids
16	20.44	Tridecane	$C_{13}H_{28}$	184.3	Alkanes
17	20.77	Undecanal	$C_{11}H_{22}O$	170.2	Aldehydes
18	24.03	β-Damascenone	$C_{13}H_{18}O$	190.3	Ketones
19	24.87	Tetradecane	$C_{14}H_{30}$	198.3	Alkanes
20	25.34	Dodecanal	$C_{12}H_{24}O$	184.3	Aldehydes
21	25.63	Caryophyllene	$C_{15}H_{24}$	204.3	Terpenoids
22	27.33	p-(n-Tetradecyloxy) phenol	$C_{20}H_{34}O_2$	306.4	Phenols
23	27.55	Geranylacetone	$C_{13}H_{22}O$	194.3	Ketones
24	27.95	2,6,10-Trimethyltridecane	$C_{16}H_{34}$	226.4	Alkanes
25	28.51	Undecanoic acid	$C_{11}H_{22}O_2$	186.2	Fatty acids
26	28.83	β-Ionone	$C_{13}H_{20}O$	192.3	Terpenoids
27	29.43	Pentadecane	$C_{15}H_{32}$	212.4	Alkanes
28	29.81	Tridecanal	$C_{13}H_{26}O$	198.3	Aldehydes
29	31.89	Caryophyllene oxide	$C_{15}H_{24}O$	220.3	Terpenoids
30	32.47	Hexadecane	$C_{16}H_{34}$	226.4	Alkanes
31	32.82	Tetradecanal	$C_{14}H_{28}O$	212.3	Aldehydes
32	34.87	Heptadecane	$C_{17}H_{36}$	240.4	Alkanes
33	35.20	Pentadecanal	$C_{15}H_{30}O$	226.4	Aldehydes
34	35.44	Methyl tetradecanoate	$C_{15}H_{30}O_2$	242.4	Esters
35	36.84	Ethyl myristate	$C_{16}H_{32}O_2$	256.4	Esters
36	36.93	Octadecane	$C_{18}H_{38}$	254.4	Alkanes
37	37.27	Hexadecanal	$C_{16}H_{32}O$	240.4	Aldehydes
38	37.81	Hexahydrofarnesyl acetone	$C_{18}H_{36}O$	268.4	Terpenoids
39	39.14	Farnesyl acetone	$C_{18}H_{30}O$	262.4	Terpenoids
40	39.27	Methyl Palmitate	$C_{17}H_{34}O_2$	270.4	Esters
41	42.07	Heneicosane	$C_{21}H_{44}$	296.5	Alkanes
42	42.13	Methyl 11,14,17-eicosatrienoate	$C_{21}H_{36}O_2$	320.5	Esters
43	44.63	Muscalure	$C_{23}H_{46}$	322.6	Alkenes
44	45.02	Tricosane	$C_{23}H_{48}$	324.6	Alkanes

3.2. HS-GC-MS Fingerprints Analysis of Volatile Components in FF

The volatile oil in twenty batches of FF samples collected in different areas were analyzed by HS-GC-MS. The HS-GC-MS chromatograms of twenty batches of FF samples and the reference fingerprint are shown in Figure 2. The HS-GC-MS chromatogram data of the FF samples were imported into the Similarity Evaluation System for Chromatographic Fingerprints of Traditional Chinese Medicine (2012 Edition) to perform common peak

alignment and similarity analysis. The reference fingerprint was established via the average method after multi-point calibration and data alignment. A total of fifteen common peaks were screened from the FF samples by full-spectrum peak matching with a 0.1 min time window, which represented the similarity among the FF samples. The fifteen common peaks were identified as linalool oxide (6), nonanal (9), undecanal (17), dodecanal (20), pentadecane (27), tridecanal (28), hexadecane (30), tetradecanal (31), heptadecane (32), pentadecanal (33), hexadecanal (37), hexahydrofarnesyl acetone (38), farnesyl acetone (39), muscalure (43), and tricosane (44) according to the identified results above. Then, the similarities between each batch of FF samples and the reference fingerprint were calculated, and the similarities (Supplementary Table S3) of the twenty batches of FF samples were between 0.706 and 0.971 (excluding S10). The similarity results indicated that the volatile components of FF samples from different origins were similar, but there still might be some internal differences. Therefore, chemometrics analysis was further employed to compare and evaluate the volatile constituents in different batches of the FF samples.

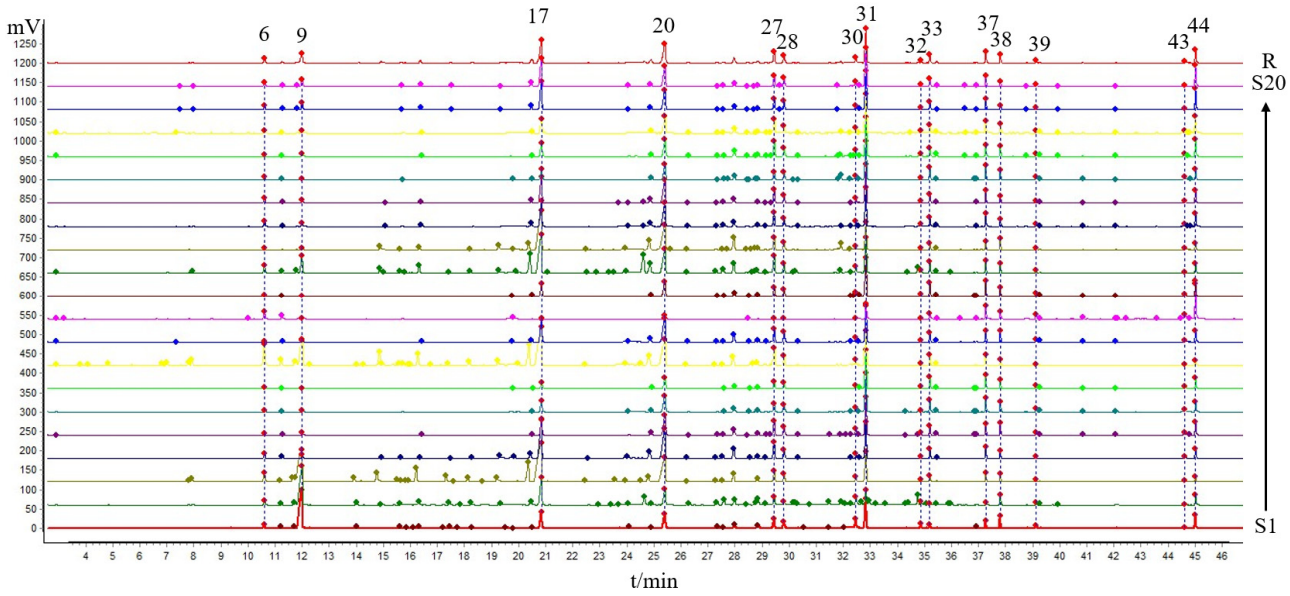


Figure 2. The HS-GC-MS fingerprint chromatograms (S1–S20) and reference fingerprint (R) of FF samples: 6 linalool oxide, 9 nonanal, 17 undecanal, 20 dodecanal, 27 pentadecane, 28 tridecanal, 30 hexadecane, 31 tetradecanal, 32 heptadecane, 33 pentadecanal, 37 hexadecanal, 38 hexahydrofarnesyl acetone, 39 farnesyl acetone, 43 muscalure, and 44 tricosane.

3.3. Chemometrics Analysis

HCA was first employed based on the peak areas of the fifteen common peaks in fingerprint to compare and reveal the relatively homogeneous clusters among the FF samples. The peak area values of fifteen common peaks in twenty batches of FF samples were imported into SPSS software. The dendrogram of HCA is presented in Figure 3A. When the Euclidean distance was 15, all FF samples were divided into four categories: S8, S10 and S20 formed three separate clusters (II, III, IV), while all the other samples were grouped into one category (I). The HCA results revealed that twenty batches of FF samples from four areas including Shandong (SD) province (S1–S3), Henan (HN) province (S4–S7), Shanxi (SX) province (S8–S16) and Hebei (HB) province (S17–S20) could not be clearly differentiated in HCA. The results indicated that the volatile components in FF samples were highly similar, and no obvious differences were observed in volatile constituents among FF samples from different production areas.

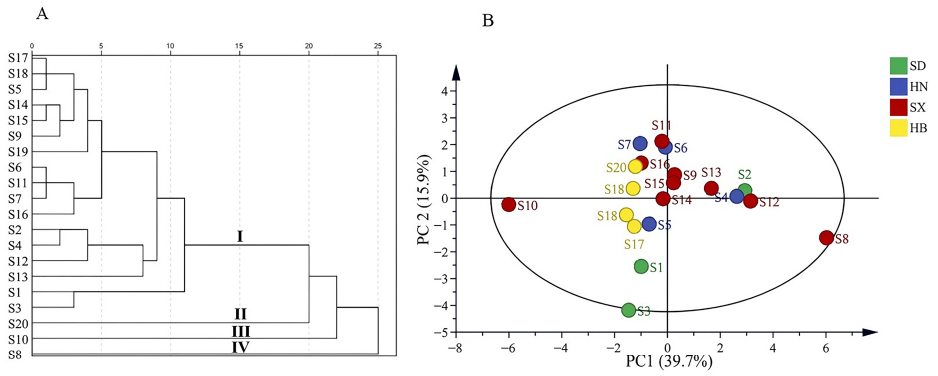


Figure 3. HCA (**A**) and PCA (**B**) of volatile components of FF samples. S1–S3 Shandong (SD) samples, S4–S7 Henan (HN) samples, S8–S16 Shanxi (SX) samples, S17–S20 Hebei (HB) samples. I FF samples except S8, S10 and S20, II S20, III S10, IV S8.

PCA is a commonly used chemometric method for analyzing and reducing dimensionality of numerical datasets, and has been widely applied to compare and discriminate the chemical compositions in herbal medicines [15]. PCA was further performed to provide more information about volatile constituents in FF samples from different producing areas. The peak areas of fifteen common peaks in fingerprint were imported into SIMCA 14.1 software for PCA. As shown in Figure 3B, the green dots, blue dots, red dots and yellow dots represented the Shandong (SD), Henan (HN), Shanxi (SX) and Hebei samples, respectively. Similar to the HCA results, most FF samples were clustered into one group. Neither PCA nor HCA could effectively distinguish and classify FF samples from different producing areas. The chemometrics results indicated that the volatile components exhibited no significant variation among the FF samples, and volatile compounds might be insufficient for identification and discrimination of FFs collected from different areas.

3.4. Chemical Characterization of Non-Volatile Components in FF by UPLC-QTOF-MS

The volatile components in FF were first analyzed by HS-GC-MS. In order to comprehensively characterize the chemical components of FF, an UPLC-QTOF-MS method was further employed to identify non-volatile compounds in FF samples. The typical total ion chromatogram of a FF sample in positive ion mode is displayed in Figure 4. A total of thirty-two non-volatile constituents were characterized and identified, including 9 lignans, 8 flavonoids, 7 organic acids, 6 phenylethanoid glycosides, 1 coumarin, and 1 other compound. Among the identified constituents, 7 compounds (chlorogenic acid, rutin, forsythoside A, (+)-pinoresinol-4-O-β-D-glucopyranoside, phillyrin, quercetin and phillygenin) were unambiguously identified by comparing with the reference standards, and the other 25 compounds were tentatively identified by comparing the retention time, accurate molecular mass and MS/MS fragment patterns with previous reports [4,6,16–19]. Detailed information including retention time, chemical formulas, measured masses and main fragment ions are listed in Table 2. The structures of non-volatile compounds identified in FF are shown in Supplementary Figure S1. According to the UPLC-QTOF-MS results, the main non-volatile components of FF were lignans, flavonoids and phenylethanoid glycosides, which were similar to those found in *Forsythia suspensa* fruit. Lignans, phenylethanoid glycosides and flavonoids are the main active constituents in *Forsythia suspensa*, such as forsythoside A, phillyrin and phillygenin [20,21]. These compounds exhibited anti-inflammatory, antioxidant, antibacterial, antivirus, anti-cancer, anti-allergy effects, etc. [22,23]. The results indicated that FF might have great development potential as functional food ingredients and medicinal materials due to its abundant bioactive components and stable quality properties.

Table 2. The information of non-volatile components identified in FF samples by UPLC-QTOF-MS.

Peak	RT (min)	Identification	Formula	Measured	MS/MS	Error (ppm)	Classification
1	2.50	p-Coumaric acid	$C_9H_8O_3$	165.0546	147.0440, 119.0483	−0.72	Organic acids
2	3.17	Vanillic acid	$C_8H_8O_4$	169.0498	151.0367, 123.0445	0.10	Organic acids
3	3.62	3,4-Diethoxybenzoic acid	$C_{11}H_{14}O_4$	211.0964	170.0606, 155.1095, 119.0490, 113.9621	−0.62	Organic acids
4	4.25	Maoliunoside	$C_{14}H_{20}O_7$	301.1276	247.1367, 189.1234,165.0888	−0.04	Phenylethanoid glycosides
5	4.31	Caffeic acid	$C_9H_8O_4$	181.0493	163.1136, 135.0625, 107.0844	0.32	Organic acids
6	4.92	Forsythoside B	$C_{22}H_{26}O_{11}$	467.1543	297.0938, 165.0511, 151.0392, 149.0574, 135.0434	−0.39	Phenylethanoid glycosides
7	5.28	Quinic acid	$C_7H_{12}O_6$	193.0703	193.0462, 176.1066, 131.9274, 103.0323	−0.45	Organic acids
8	5.70	Chlorogenic acid *	$C_{16}H_{18}O_9$	355.1008	191.0364, 163.0387, 145.0280, 135.0432, 117.0338	−0.38	Organic acids
9	7.45	6,7-Dimethoxycoumarin	$C_{11}H_{10}O_4$	207.0648	207.0648, 153.0768	−2.12	Coumarins
10	7.90	p-Coumaroyl quinic acid	$C_{16}H_{18}O_8$	339.1073	147.0442, 119.0489	−0.48	Organic acids
11	8.32	Demethylphillyrin	$C_{26}H_{32}O_{11}$	521.2013	359.1472, 341.8462, 324.1222, 137.0549	−1.43	Lignans
12	8.40	Pinoresinol-4-O-glucoside	$C_{26}H_{32}O_{11}$	521.2017	360.1537, 153.0386, 138.0622	−1.69	Lignans
13	13.66	Hyperoside	$C_{21}H_{20}O_{12}$	465.1032	303.0489, 285.0394, 257.0443, 229.0491	−1.64	Flavonoids
14	14.19	Quercitrin	$C_{21}H_{20}O_{12}$	465.1028	303.0492, 285.0386, 257.0430, 229.0489	0.24	Flavonoids
15	14.26	Rutin *	$C_{27}H_{30}O_{16}$	611.1629	465.1008, 303.0499, 257.0441, 147.0646, 129.0548	−0.79	Flavonoids
16	14.28	Arctigenin	$C_{21}H_{24}O_6$	373.1646	295.1324, 137.0594	−2.26	Lignans
17	14.30	Astragalin	$C_{27}H_{30}O_{16}$	611.1607	285.0396, 165.0181	0.51	Flavonoids
18	15.30	Calceolarioside A	$C_{23}H_{26}O_{11}$	479.1548	326.0921, 163.0389	0.82	Phenylethanoid glycosides
19	15.39	Forsythoside A *	$C_{29}H_{36}O_{15}$	625.2119	479.1431, 462.1513, 445.7629, 325.0910,179.0693, 163.0772, 136.0431	−2.47	Phenylethanoid glycosides
20	15.40	Calceolarioside B	$C_{23}H_{26}O_{11}$	479.1548	179.0689, 163.0389, 153.1263, 137.0579	−1.88	Phenylethanoid glycosides
21	15.42	Isorhamnetin	$C_{16}H_{12}O_7$	316.0541	-	−2.73	Flavonoids
22	15.45	Acteoside	$C_{29}H_{36}O_{15}$	625.2127	325.0882, 163.0382, 145.0273	−1.99	Phenylethanoid glycosides
23	17.19	Kaempferol	$C_{15}H_{10}O_6$	287.0543	213.0504, 185.0595, 165.4002	−0.25	Flavonoids
24	18.20	(+)-Pinoresinol-4-O-β-D-glucopyranoside *	$C_{26}H_{32}O_{11}$	521.2017	360.1537, 153.0531, 138.0622	−0.08	Lignans
25	20.19	Kaempferol-3-O-rutinoside	$C_{27}H_{30}O_{15}$	595.1657	287.0543, 240.133, 164.9136	−1.96	Flavonoids
26	28.05	Epipinoresinol	$C_{20}H_{22}O_6$	359.1489	341.1403, 235.1062, 205.0621, 151.0363, 125.0576	−0.72	Lignans

Table 2. *Cont.*

Peak	RT (min)	Identification	Formula	Measured	MS/MS	Error (ppm)	Classification
27	28.96	Citral	$C_{10}H_{16}O$	153.1274	139.0934	−1.9	Others
28	31.44	Phillyrin *	$C_{27}H_{34}O_{11}$	557.1985	395.1458, 379.1134, 370.0753, 356.1728, 309.0894, 163.0365	0.23	Lignans
29	32.00	Arctiin	$C_{27}H_{34}O_{11}$	557.1993	176.9592, 163.0365, 151.0743, 137.0594	−0.34	Lignans
30	34.14	Quercetin *	$C_{15}H_{10}O_7$	303.0499	303.0497, 285.0391, 257.0448, 229.0492	0.11	Flavonoids
31	34.81	Pinoresinol	$C_{20}H_{22}O_6$	359.1489	341.1404, 291.1023, 270.0795, 187.0774, 137.0592	0.08	Lignans
32	36.99	Phillygenin *	$C_{21}H_{24}O_6$	373.1646	355.1526, 337.1444	−0.38	Lignans

* Compounds identified by reference standards.

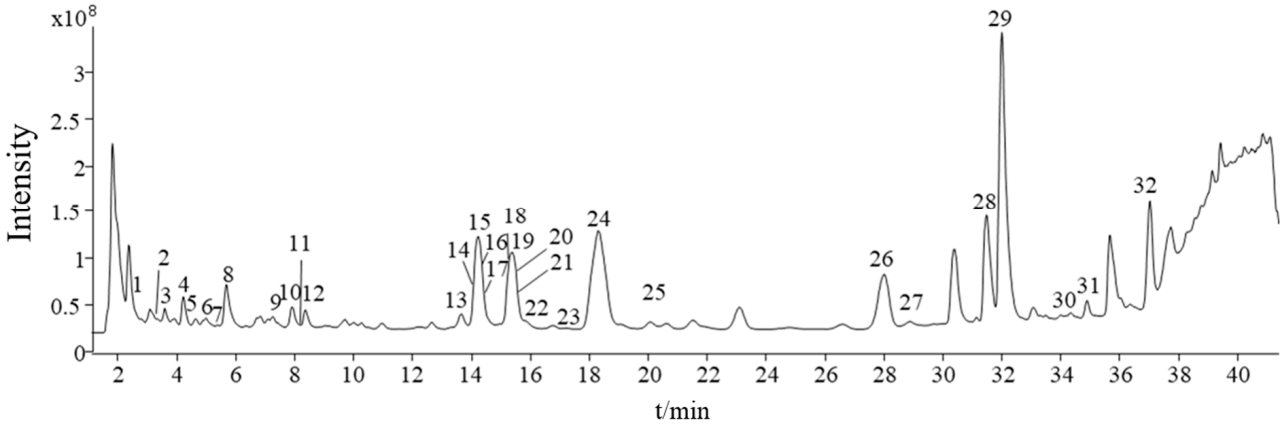


Figure 4. The total ion chromatogram of non-volatile components of FF by UPLC-QTOF-MS in positive mode. The peak numbers are consistent with the compound numbers presented in Table 2.

3.4.1. Identification of Phenylethanoid Glycosides

Phenylethanoid glycosides are one of the main bioactive components in FF. A total of 6 phenylethanoid glycosides, including maoliunoside (4), forsythoside B (6), calceolarioside A (18), forsythoside A (19), calceolarioside B (20), acteoside (22) were initially characterized in FF. For instance, the molecular formula of compound 19 was $C_{29}H_{36}O_{15}$ based on its precursor ion at m/z 625.2119 $[M + H]^+$. The MS/MS fragment ions were exhibited at m/z 462.1513 $[M + H\text{-}C_9H_6O_3]^+$, 136.0431 $[M + H\text{-}C_9H_6O_3\text{-}C_6H_{10}O_4\text{-}C_6H_{10}O_5\text{-}H_2O]^+$. Compound 19 was identified as forsythoside A by characteristic fragmentation patterns and literature [23]. The MS/MS spectrometry and fragmentation pathway of forsythoside A are shown in Supplementary Figure S2A.

3.4.2. Identification of Flavonoids

A total of 8 flavonoids, including hyperoside (13), quercitrin (14), rutin (15), astragalin (17), isorhamnetin (21), kaempferol (23), kaempferol-3-O-rutinoside (25) quercetin (30) were initially identified in FF. Rutin could be an example for identification of flavonoids in FF. Rutin exhibited the precursor ion at m/z 611.1629$[M + H]^+$, and the molecular formula was $C_{27}H_{30}O_{16}$. The MS/MS fragment ions were exhibited at m/z 465.1008 $[M + H\text{-}C_6H_{10}O_4]^+$, m/z 303.0499 $[M + H\text{-}C_6H_{10}O_4\text{-}C_6H_{10}O_5]^+$ by loss of glucose and rhamnose. Further fragmentation of the aglycone occurred via RDA rearrangement, followed by losses of CO_2 and H_2O, producing characteristic fragment ions at m/z 257.0441 and 129.0548. The MS/MS spectrometry and fragmentation pathway of rutin are shown in Supplementary Figure S2B.

3.4.3. Identification of Organic Acids

A total of 7 organic acid including p-coumaric acid (1), vanillic acid (2), 3,4-diethoxybenzoic acid (3), caffeic acid (5), quinic acid (7), chlorogenic acid (8), and p-coumaroyl quinic acid (10) were initially characterized in FF. For example, the molecular formula of compound 8 was $C_{16}H_{18}O_9$ and the precursor ion was m/z 355.1008$[M + H]^+$. In MS/MS spectrometry, the cleavage of the ester bond forms a quinic acid fragment with m/z 191.0364 $[M + H\text{-caffeoyl}]^+$ and a caffeic acid fragment. The quinic acid fragment further loses CH_2O, resulting in m/z 163.0387 $[\text{quinic acid-}CH_2O + H]^+$. Meanwhile, the caffeic acid fragment loses a molecule of CO_2, forming m/z 135.0432 $[\text{quinic acid-}CO_2 + H]^+$. Compound 8 was identified as chlorogenic acid by fragmentation ions and literature [24], and the MS/MS spectrometry and fragmentation pathway of chlorogenic acid are shown in Supplementary Figure S2C.

3.4.4. Identification of Lignans

Lignans are also the main active constituents in FF. In the present work, 9 lignans such as demethylphillyrin (11), pinoresinol-4-O-glucoside (12), arctigenin (16), (+)-pinoresinol-4-O-β-D-glucopyranoside (24), epipinoresinol (26), phillyrin (28), arctiin (29), pinoresinol (31), phillygenin (32) were initially identified in FF. For instance, the molecular formula of compound 28 was $C_{27}H_{34}O_{11}$ and the precursor ion was m/z 557.1985$[M + Na]^+$. In MS/MS spectrometry, the loss of a sugar moiety followed by hydrogen rearrangement generated a fragment ion at m/z 395.1458$[M + Na\text{-}C_6H_{10}O_5]^+$. Alternatively, the loss of $C_6H_{10}O_6$ produced a fragment ion at m/z 379.1134$[M + Na\text{-}C_6H_{10}O_6]^+$, and the removal of two five-membered rings along with the connected methoxyphenyl group resulted in a fragment ion at m/z 309.0894$[M + Na\text{-}C_{14}H_{16}O_4]^+$. This fragment could further lose a phenyl ring to form another fragment ion at m/z 185.0416$[M + Na\text{-}C_{14}H_{16}O_4\text{-}C_7H_8O_2]^+$. According to the literature and fragmentation behaviors, compound 28 was identified as phillyrin [25]. The MS/MS spectrometry and fragmentation pathway of phillyrin are shown in Supplementary Figure S2D.

3.5. HPLC Fingerprint Analysis of Non-Volatile Components in FF

Chemical fingerprint is a powerful tool to characterize complex chemical systems and has been extensively used for phytochemical profile and quality evaluation of herbal medicines and plant materials. Following the qualitative analysis of non-volatile compounds in FF by UPLC-QTOF-MS, the HPLC fingerprint was further applied for chemical profiling and quality assessment of FF.

3.5.1. Optimization of HPLC Condition Conditions

To achieve rapid and efficient separation of FF samples, the HPLC conditions such as mobile phases (methanol-aqueous, acetonitrile-aqueous, methanol-aqueous, and acetonitrile-aqueous formic acid), flow rates (0.2 mL/min, 0.3 mL/min and 0.4 mL/min), and column temperatures (20 °C, 30 °C and 40 °C). The results indicated that the gradient elution system of acetonitrile-aqueous formic acid exhibited the optimal separation performance for target analytes, and column temperature of 30 °C and flow rate of 0.3 mL/min were adopted in this study.

3.5.2. HPLC Fingerprint Analysis

A total of twenty batches of FF samples collected from four main production areas were analyzed by the optimized HPLC condition. The HPLC fingerprint of FF was established based on the analysis of twenty batches of FF samples for systematic pattern recognition and similarity evaluation. The HPLC chromatograms of twenty batches of FF samples and the reference fingerprint are shown in Figure 5. By automatic peak matching, seven characteristic peaks with good resolution and separation were selected as the common peaks of FF samples. These seven common peaks were identified based on the above UPLC-QTOF-MS results and comparison with authentic reference standards: chlorogenic acid (8), rutin (15), forsythoside A (19), (+)-pinoresinol-4-O-β-D-glucopyranoside (24), phillyrin (28), quercetin (30) and phillygenin (32).

The similarity evaluation of the twenty batches of FF was calculated by similarity evaluation system of chromatographic fingerprints of Traditional Chinese Medicine, and the results are shown in Supplementary Table S4. The similarity of FF samples from different producing areas was in the range of 0.853–1.000, indicating that the main non-volatile constituents in FF were similar. The established HPLC fingerprint method had good precision, stability, repeatability and accuracy, and could provide a reliable analytical tool for quality control of FF and FF-derived products.

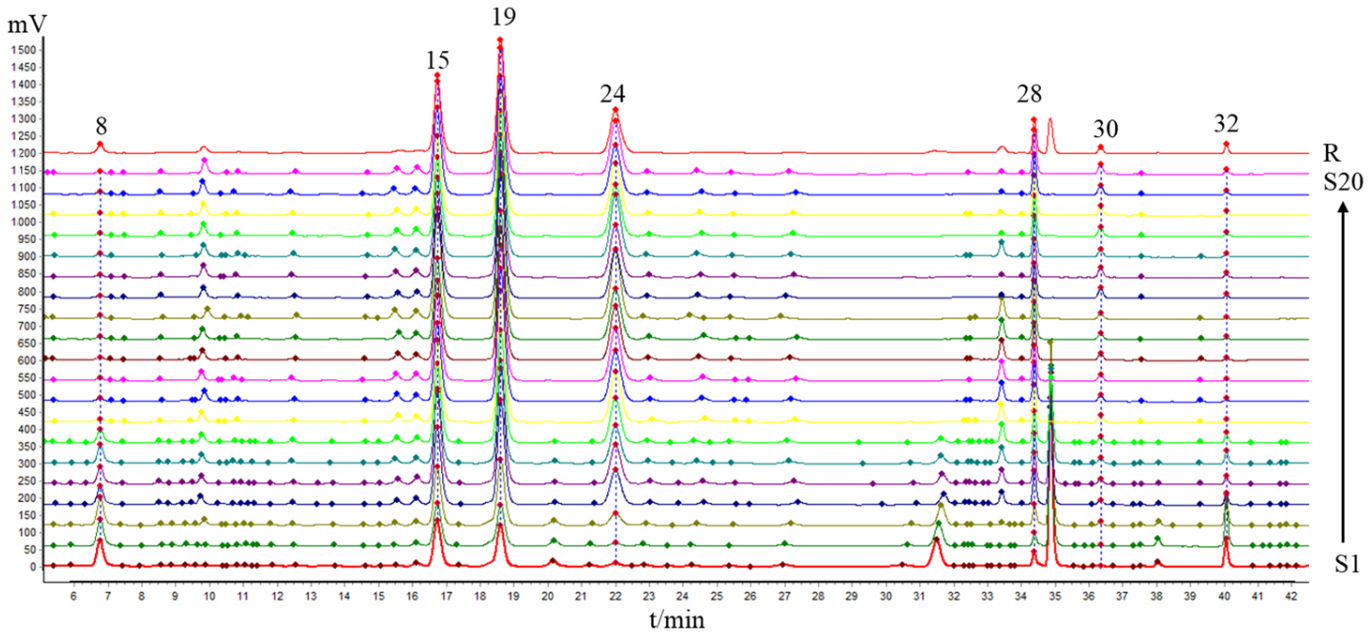


Figure 5. The HPLC fingerprint chromatograms (S1–S20) and reference fingerprint (R) of FF samples. 8 chlorogenic acid, 15 rutin, 19 forsythoside A, 24 (+)-pinoresinol-4-O-β-D-glucopyranoside, 28 phillyrin, 30 quercetin, 32 phillygenin.

3.6. Quantitative Analysis of Seven Non-Volatile Components in FF

For further quality evaluation, the compositions of bioactive compounds in FF, as well as the contents of seven non-volatile compounds identified in HPLC fingerprint including chlorogenic acid, rutin, forsythoside A, (+)-pinoresinol-4-O-β-D-glucopyranoside, phillyrin, quercetin and phillygenin, were simultaneously determined by HPLC-DAD [26,27].

3.6.1. Method Validation

The linearity, precision, repeatability, stability and accuracy of the quantitative analysis method were validated. Six level concentrations of working standard solutions were analysis to evaluate the linearity. The calibration curves of the seven compounds were obtained by regression analysis with the peak area of analytes as Y and the concentration as X. The calibration curves of the seven compounds showed a good linear relationship ($r^2 \geq 0.9992$) within the linearity range. The signal-to-noise ratios of 10 and 3 were defined individually as the limits of quantification (LOQ) and limits of detection (LOD), and the LOQ and LOD of the seven compounds ranged from 0.020 μg/mL to 0.180 μg/mL and from 0.013 μg/mL to 0.110 μg/mL, respectively. The precision of the quantitative method was analyzed by intra- and inter-day variabilities. The same sample was analyzed in six replicates in one day or in triplicate for three consecutive days. The relative standard deviations (RSDs) of intra- and inter-day precisions of the seven compounds were less than 1.7% and 2.6%, respectively. The repeatability was checked by extracting and analyzing the same sample in six replicates, and the RSD of repeatability were less than 2.8%. To test the stability, the sample was stored at room temperature and tested by replicate analysis at 0, 2, 4, 8, 12 and 24 h. The stability presented as RSD was less 2.8%. The accuracy of the quantitative analysis method was assessed by recovery experiments. Seven reference compounds at known concentrations were added to 0.10 g sample, then the sample was extracted and analyzed using the established HPLC-DAD method. The accuracy presented as recoveries of the seven compounds were in the range of 99.20–103.13%, with RSD less than 2.7%. The results of the method validation (Supplementary Table S5) showed that the

quantitative method was precise, stable, reproducible, accurate, and appropriate for the simultaneous determination of 7 non-volatile components in FF samples.

3.6.2. Quantitative Analysis of Seven Non-Volatile Components in FF Samples

The validated quantitative method was applied to simultaneously determine 7 non-volatile compounds, including chlorogenic acid, rutin, forsythoside A, (+)-pinoresinol-4-O-β-D-glucopyranoside, phillyrin, quercetin and phillygenin in twenty batches of FF samples. The representative HPLC chromatograms of the mixed reference compounds and FF samples are shown in Figure 6. The contents of the seven non-volatile constituents in FF were calculated and listed in Table 3. The contents of chlorogenic acid, rutin, forsythoside A, (+)-pinoresinol-4-O-β-D-glucopyranoside, phillyrin, quercetin and phillygenin in FF samples collected from different producing areas were 1.12–6.29 mg/g, 20.95–51.38 mg/g, 12.25–46.89 mg/g, 1.93–44.70 mg/g, 3.28–9.31 mg/g, 0.19–1.45 mg/g and 0.24–5.79 mg/g, respectively. Forsythoside A is the characteristic marker components of *Forsythia suspensa* fruit with hepatoprotective and anti-inflammatory pharmacological effects [28,29]. FF contains relatively abundant forsythoside A with contents in the ranges of 12.25 ± 0.02 to 46.89 ± 0.66 mg/g. Additionally, rutin and (+)-pinoresinol-4-O-β-D-glucopyranoside are also abundant in FF samples with the contents ranging from 20.95 ± 0.12 to 51.38 ± 0.64 mg/g and 1.93 ± 0.02 to 44.70 ± 0.63 mg/g, respectively. These two compounds exert multiple pharmacological effects, such as anti-inflammatory, antibacterial, antioxidant, anti-hepatic injury and antidiabetic activities [30,31]. The quantitative results suggested that FF contains several bioactive components that possess various activities, and provided a theoretical basis for its development and application as a functional dietary supplement.

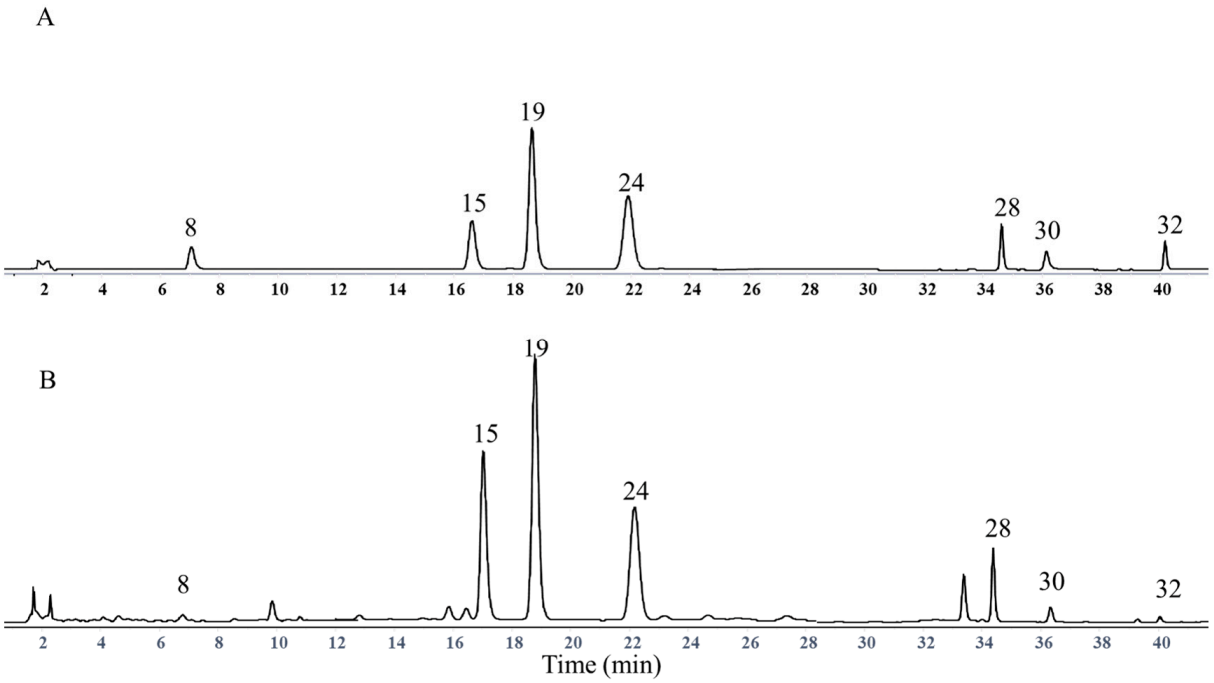


Figure 6. The HPLC chromatograms of mixed reference compounds (**A**) and FF sample (**B**). 8 chlorogenic acid, 15 rutin, 19 forsythoside A, 24 (+)-pinoresinol-4-O-β-D-glucopyranoside, 28 phillyrin, 30 quercetin, 32 phillygenin.

Table 3. The contents of seven non-volatile components in twenty batches of FF samples (mg/g, n = 3).

NO.	Chlorogenic Acid	Rutin	Forsythoside A	(+)-Pinoresinol-4-O-β-D-Glucopyranoside	Phillyrin	Quercetin	Phillygenin
S1	5.92 ± 0.05	23.01 ± 0.54	12.25 ± 0.02	2.95 ± 0.09	3.41 ± 0.10	0.19 ± 0.01	3.53 ± 0.12
S2	6.03 ± 0.05	20.95 ± 0.12	12.40 ± 0.58	1.93 ± 0.02	3.28 ± 0.04	0.26 ± 0.02	5.79 ± 0.02
S3	6.29 ± 0.02	28.58 ± 0.18	20.19 ± 0.07	8.33 ± 0.19	4.66 ± 0.03	0.53 ± 0.02	3.99 ± 0.07
S4	3.71 ± 0.20	38.22 ± 0.10	29.97 ± 0.13	23.03 ± 0.30	6.81 ± 0.41	0.76 ± 0.01	1.38 ± 0.13
S5	3.87 ± 0.10	44.15 ± 0.37	33.05 ± 0.59	25.42 ± 0.84	6.56 ± 0.12	0.75 ± 0.03	1.08 ± 0.05
S6	3.64 ± 0.04	37.04 ± 0.55	29.55 ± 0.30	25.02 ± 0.30	6.14 ± 0.06	0.82 ± 0.02	1.82 ± 0.09
S7	3.04 ± 0.01	38.38 ± 0.37	31.84 ± 0.02	28.64 ± 0.01	6.75 ± 0.02	0.99 ± 0.03	1.38 ± 0.01
S8	1.32 ± 0.02	40.68 ± 0.32	38.89 ± 0.08	34.16 ± 0.43	7.80 ± 0.06	0.97 ± 0.02	0.36 ± 0.03
S9	1.30 ± 0.02	39.87 ± 0.52	39.60 ± 0.71	34.58 ± 0.81	8.24 ± 0.11	0.97 ± 0.01	0.42 ± 0.01
S10	1.26 ± 0.01	42.95 ± 0.09	39.28 ± 0.01	34.37 ± 0.02	7.45 ± 0.01	0.90 ± 0.02	0.33 ± 0.01
S11	1.28 ± 0.01	39.85 ± 0.37	38.37 ± 0.63	35.00 ± 0.27	7.79 ± 0.02	1.03 ± 0.02	0.39 ± 0.01
S12	1.27 ± 0.02	39.30 ± 0.12	38.18 ± 0.32	34.69 ± 0.65	8.04 ± 0.11	0.95 ± 0.02	0.42 ± 0.02
S13	1.42 ± 0.02	51.38 ± 0.64	46.89 ± 0.66	44.70 ± 0.63	8.33 ± 0.14	0.83 ± 0.02	0.24 ± 0.01
S14	1.24 ± 0.01	42.76 ± 0.07	35.11 ± 0.05	32.00 ± 0.05	7.34 ± 0.01	1.43 ± 0.04	0.60 ± 0.01
S15	1.25 ± 0.01	41.29 ± 0.13	36.72 ± 0.05	34.73 ± 0.03	8.02 ± 0.01	1.45 ± 0.02	0.61 ± 0.01
S16	1.23 ± 0.02	40.43 ± 0.34	36.19 ± 0.15	39.22 ± 0.75	8.63 ± 0.05	1.20 ± 0.03	0.47 ± 0.01
S17	1.12 ± 0.05	38.85 ± 0.04	35.57 ± 0.06	34.43 ± 0.06	8.38 ± 0.01	1.35 ± 0.02	0.53 ± 0.01
S18	1.18 ± 0.01	38.85 ± 0.10	35.72 ± 0.09	34.16 ± 0.12	8.50 ± 0.01	1.43 ± 0.01	0.55 ± 0.01
S19	1.18 ± 0.01	43.06 ± 0.05	34.40 ± 0.12	32.71 ± 0.06	8.61 ± 0.01	1.44 ± 0.03	0.58 ± 0.01
S20	1.22 ± 0.02	43.67 ± 0.41	35.35 ± 0.15	34.26 ± 0.22	9.31 ± 0.06	1.44 ± 0.01	0.63 ± 0.01

https://doi.org/10.3390/pr14213407

3.6.3. Chemometrics Analysis

The quantitative results indicated that the types of bioactive components were consistent among FF samples collected in different areas, but the contents showed obvious differences. To further compare and evaluate the quality of FF samples, HCA and PCA were performed based on the characteristics of the contents of the seven non-volatile components.

The contents of seven non-volatile compounds in twenty batches of FF samples were used as variables and imported into SPSS software. After standardization, inter-group linkage and square Euclidean distance method were employed for HCA. As shown in Figure 7A, when the Euclidean distance was 5, the FF from four producing areas could be clustered into three categories. FF samples from Shanxi and Hebei (S8–S20) belonged to one cluster (I), samples from Henan (S4–S7) to the second cluster (II), and samples from Shandong (S1–S3) to the third cluster (III). The HCA results showed that the contents of main non-volatile components in FF samples collected in different production areas were different.

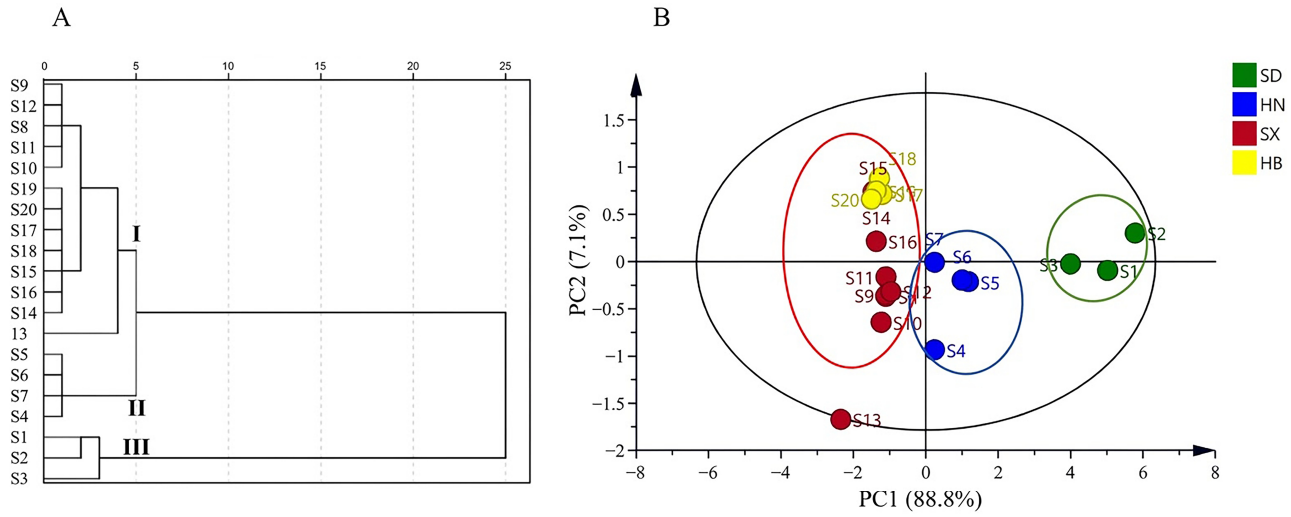


Figure 7. HCA (**A**) and PCA (**B**) of seven non-volatile components in FF samples. S1–S3 Shandong (SD) samples, S4–S7 Henan (HN) samples, S8–S16 Shanxi (SX) samples, S17–S20 Hebei (HB) samples. I S8-S20, II S4-S7, III S1-S3.

PCA was further employed to characterize the differences about non-volatile constituents in FF samples. The contents of the seven non-volatile compounds were imported into SIMCA 14.1 software, then the data were preprocessed by unit variance (UV) scaling. In the PCA model, the contribution rates of first principal components (PC1) and second principal components (PC2) were 88.8% and 7.1%, and the first two principal components explained 95.9% of total variance. The first two principal components (PC1 and PC2) could comprehensively reflect the difference in FF samples. The variance cumulative interpretation ability parameter R^2X and the predictive ability parameter Q^2 were 0.959 and 0.852, respectively, demonstrating that the established PCA model had good discrimination and predictive ability. The PCA score plot is displayed in Figure 7B. Similar to the results of HCA, PCA results showed that FF samples divided into three groups: Shandong samples (S1–S3), Henan samples (S4–S7), Shanxi and Hebei samples (S8–S20). Both HCA and PCA results indicated that the non-volatile constituents existed differences among FF samples from different origins, which might be related to regional environmental and climatic conditions [32,33].

Based on the quantitative and chemometrics analysis results of FF samples from different producing areas, the contents of seven non-volatile compounds in twenty batches of FF were further compared. As shown in Figure 8, there were obvious differences in

the average contents of the seven components among FF samples from different origins. Notably, the contents of rutin and forsythoside A in Shandong samples were significantly lower than those in Shanxi, Henan and Hebei samples. Lignans including (+)-pinoresinol-4-O-β-D-glucopyranoside and phillyrin showed relatively low concentrations in Shandong and Henan samples. Quercetin exhibited a relatively high level in Hebei samples, while its concentration reached the minimum in Shandong samples.

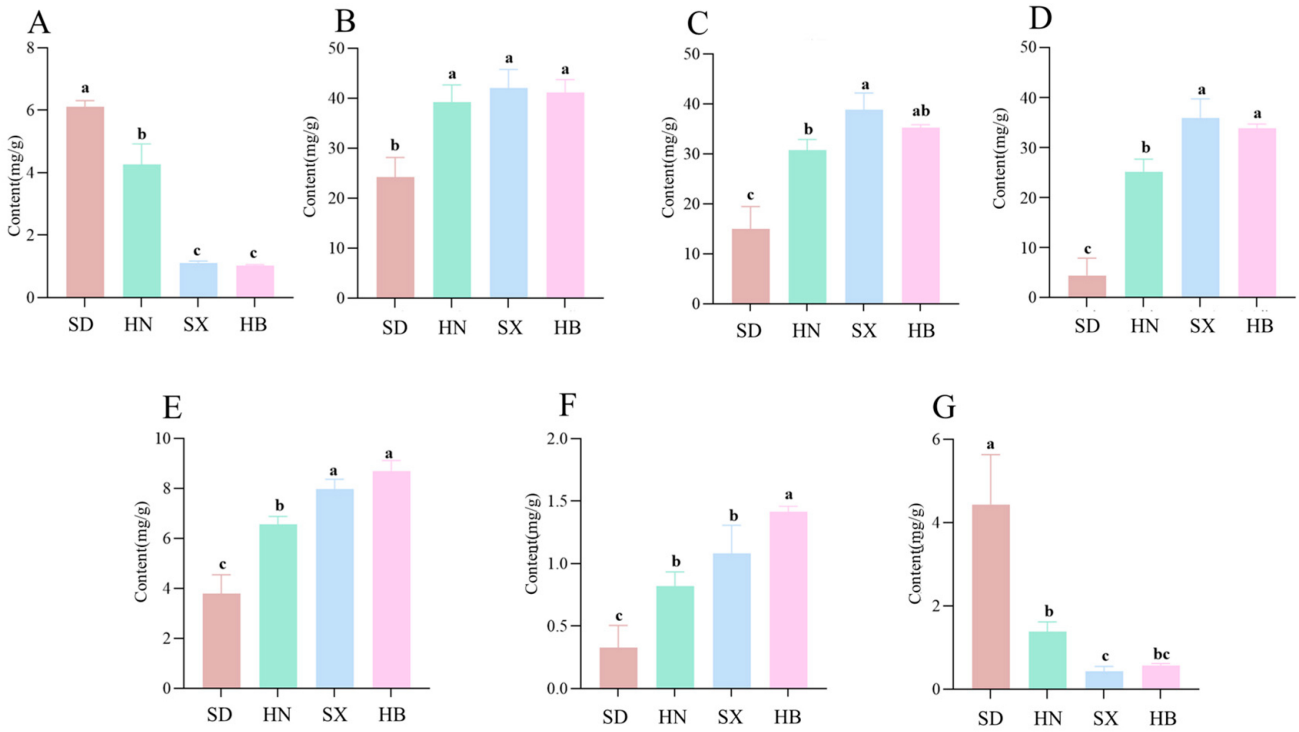


Figure 8. The average contents of seven non-volatile compounds in FF samples collected from different production areas. (**A**) Chlorogenic acid, (**B**) rutin, (**C**) forsythoside A, (**D**) (+)-pinoresinol-4-O-β-D-glucopyranoside, (**E**) phillyrin, (**F**) quercetin, (**G**) phillygenin. Different letters indicate significant differences in the contents of the components among different samples ($p < 0.05$); the same letter indicates no significant difference ($p > 0.05$).

4. Conclusions

In this present work, HS-GC-MS, UPLC-QTOF-MS and HPLC-DAD method were applied to characterize the volatile and non-volatile constituents in FF. A total of 44 volatile compounds including 12 aldehydes, 11 alkanes, 8 terpenoids, 4 ketones, 4 esters, 2 organic acids, 1 alcohol, and 1 alkene were identified by HS-GC-MS, and 32 non-volatile compounds including 9 lignans, 8 flavonoids, 7 organic acids, 6 phenylethanoid glycosides, 1 coumarin, and 1 other compound were characterized by UPLC-QTOF-MS. Furthermore, GC-MS fingerprint and chemometric analysis was employed to evaluate and compare the volatile compositions in FF samples from different production areas. The chemometrics results revealed that the volatile components displayed insignificant variation among FF samples, and the volatile compositions were similar in FF samples collected from different production areas. Then, an HPLC fingerprint was also established to assess the non-volatile components of FF samples. A total of seven common peaks in the fingerprint of FF were identified, including chlorogenic acid, rutin, forsythoside A, (+)-pinoresinol-4-O-β-D-glucopyranoside, phillyrin, quercetin and phillygenin. The established HPLC fingerprint method had good specificity and could be used for the quality evaluation of FF. Moreover, a validated HPLC-DAD method was employed to simultaneous quantitative analysis of seven compounds in twenty batches of FF samples. The quantitative

and chemometrics results showed obvious differences in non-volatile constituents in FF samples from different origins. In conclusion, this study provided dependable and credible analytical methods for the chemical profiling and quality evaluation of FF samples, and offered fundamental chemical evidence to support the further development and utilization of FF. Future research is recommended to focus on biological activities of FF, which will help verify its prospective applications in functional food and pharmaceutical fields.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/pr14213407/s1. Figure S1: The chemical structures of compounds identified in FF by UPLC-QTOF-MS. The numbers are consistent with the compound numbers presented in Table 1. Figure S2: The MS/MS spectrum and proposed fragmentation pattern of forsythoside A (A), rutin (B), chlorogenic acid (C) and phillyrin (D). Table S1: The information of twenty batches of FF samples (S1–S20). Table S2: The relative contents (%) of the identified volatile compounds by HS-GC-MS in twenty batches of FF samples (S1–S20). Table S3: HS-GC-MS fingerprint similarity value of FF samples. Table S4: HPLC fingerprint similarity value of FF samples. Table S5: The method validation, including calibration curves, linear range, LOQ, LOD, precision, repeatability, stability and recovery of seven non-volatile components determined in FF samples.

Author Contributions: Conceptualization, L.G. and D.Z.; methodology, L.W.; validation, J.L., J.Z. and Y.R.; investigation, J.L. and J.Z.; resources, Y.Z.; data curation, J.L. and J.Z.; writing—original draft preparation, J.L. and J.Z.; writing—review and editing, L.G.; visualization, J.L. and J.Z.; supervision, Y.Z. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The original contributions presented in this study are included in the article/Supplementary Materials. Further inquiries can be directed to the corresponding authors.

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

Abbreviations

The following abbreviations are used in this manuscript:

FF	*Forsythia suspensa* flowers
HS-GC-MS	Headspace gas chromatography–mass spectrometry
UPLC-QTOF-MS	Ultra-performance liquid chromatography–quadrupole time-of-flight mass spectrometry
HPLC-DAD	High-performance liquid chromatography–diode array detection
HCA	Hierarchical clustering analysis
PCA	Principal component analysis

References

1. Zhao, Y.; Peng, Q.; Chang, L.; Wang, Z.; Lin, Z.; Shi, J.; Dong, C. Metabolomics and sensory evaluation combined analysis reveal the effect of processing methods and different forms on the flavor of rose herbal tea. *npj Sci. Food* **2025**, *9*, 45. [CrossRef]
2. Wang, Z.; Xia, Q.; Liu, X.; Liu, W.; Huang, W.; Mei, X.; Luo, J.; Shan, M.; Lin, R.; Zou, D.; et al. Phytochemistry, pharmacology, quality control and future research of *Forsythia suspensa* (Thunb.) Vahl: A review. *J. Ethnopharmacol.* **2018**, *210*, 318–339. [CrossRef]
3. Tokar, M.; Klimek, B. The content of lignan glycosides in Forsythia flowers and leaves. *Acta Pol. Pharm.* **2004**, *61*, 273–278.
4. Mu, Y.; Liu, B.; Zhang, X.; Zhang, Y.; Wei, J.; Wang, X.Q.; Chen, Y.W.; Cui, X.L.; Pan, Y.N.; Sun, Y. Analysis of constituents in different parts of Forsythia suspensa by UPLC-Q-TOF-MS and evaluation of their anti-inflammatory activity. *Zhongguo Zhong Yao Za Zhi* **2024**, *49*, 968–980. [CrossRef]
5. Xie, Q.; Luo, J.; Liang, L.; Su, W.; Lyu, M.; Peng, C.; Li, B.; Wang, W.; Yuan, H. Effects of different drying methods on the flavor characteristics and chemical profile of *Forsythia suspensa* flowers using electronic sensors and mass spectrometry. *Foods* **2026**, *15*, 1757. [CrossRef]

6. Ji, Y.L.; Feng, X.; Chang, Y.Q.; Zheng, Y.G.; Hou, F.J.; Zhang, D.; Guo, L. Chemical characterization of different parts of *Forsythia suspensa* and α-glucosidase and pancreatic lipase inhibitors screening based on UPLC-QTOF-MS/MS and plant metabolomics analysis. *Arab. J. Chem.* **2024**, *17*, 105723. [CrossRef]
7. Kim, T.W.; Shin, J.S.; Chung, K.S.; Lee, Y.G.; Baek, N.I.; Lee, K.T. Anti-inflammatory mechanisms of koreanaside A, a lignan isolated from the flower of *Forsythia koreana*, against LPS-induced macrophage activation and DSS-induced colitis mice: The crucial role of AP-1, NF-κB, and JAK/STAT signaling. *Cells* **2019**, *8*, 1163. [CrossRef]
8. Lee, Y.G.; Jang, S.A.; Seo, K.H.; Gwag, J.E.; Kim, H.G.; Ko, J.H.; Ji, S.A.; Kang, S.C.; Lee, D.Y.; Baek, N.I. New lignans from the flower of *Forsythia koreana* and their suppression effect on VCAM-1 expression in MOVAS cells. *Chem. Biodivers.* **2018**, *15*, e1800026. [CrossRef]
9. Queiroz, E.F.; Guillarme, D.; Wolfender, J.L. Advanced high-resolution chromatographic strategies for efficient isolation of natural products from complex biological matrices: From metabolite profiling to pure chemical entities. *Phytochem. Rev.* **2024**, *23*, 1415–1442. [CrossRef]
10. Gowda, G.A.; Djukovic, D. Overview of mass spectrometry-based metabolomics: Opportunities and challenges. *Methods Mol. Biol.* **2014**, *1198*, 3–20. [CrossRef]
11. Tang, D.; Quan, C.; Huang, S.; Wei, F. Integrating LC-MS and HS-GC-MS for the metabolite characterization of the Chinese medicinal plant *Platostoma palustre* under different processing methods. *Front. Nutr.* **2023**, *10*, 1181942. [CrossRef]
12. Liu, X.; Jiang, W.; Su, M.; Sun, Y.; Liu, H.; Nie, L.; Zang, H. Quality evaluation of traditional Chinese medicines based on fingerprinting. *J. Sep. Sci.* **2020**, *43*, 6–17. [CrossRef]
13. Feng, X.; Shi, H.; Yang, G.Y.; Chang, Y.Q.; Zhang, D.; Zheng, Y.G.; Guo, L. Dynamic changes of volatile components in *Forsythia suspensa* at different harvest periods based on GC-MS and chemometrics analysis. *Zhongguo Zhong Yao Za Zhi* **2022**, *47*, 54–61. [CrossRef]
14. Diao, X.; Ma, X.; Qin, L.; Li, N.; Feng, S.; Li, J. GC-IMS was employed to analyze volatile components in *Forsythia suspensa* at different harvesting stages and in various parts. *J. Chromatogr. A* **2025**, *1763*, 466450. [CrossRef]
15. Shi, H.; Chang, Y.Q.; Feng, X.; Yang, G.Y.; Zheng, Y.G.; Zheng, Q.; Zhang, L.L.; Zhang, D.; Guo, L. Chemical comparison and discrimination of two plant sources of Angelicae dahuricae Radix, *Angelica dahurica* and *Angelica dahurica* var. *formosana*, by HPLC-Q/TOF-MS and quantitative analysis of multiple components by a single marker. *Phytochem. Anal.* **2022**, *33*, 776–791. [CrossRef]
16. Wang, X.; Jia, Q.; Yao, X.; Yang, L.; Pei, K.; Guo, L.; Guo, Y.; Yang, Y.; Qin, N. Analysis of *Forsythia Suspensa* fruit and leaf extracts using UHPLC-Q-Exactive-Orbitrap/MS: In vivo antioxidant activity on D-galactose-induced aging mice. *Food Chem.* **2025**, *462*, 141002. [CrossRef]
17. Liu, L.; Sun, Y.; Wen, C.; Jiang, T.; Tian, W.; Xie, X.; Cui, X.; Lu, R.; Feng, J.; Jin, A.; et al. Metabolome analysis of genus Forsythia related constituents in *Forsythia suspensa* leaves and fruits using UPLC-ESI-QQQ-MS/MS technique. *PLoS ONE* **2022**, *17*, e0269915. [CrossRef]
18. Zhou, M.; Huo, J.; Wang, C.; Wang, W. UPLC/Q-TOF MS screening and identification of antibacterial compounds in *Forsythia suspensa* (Thunb.) Vahl leaves. *Front. Pharmacol.* **2022**, *12*, 704260. [CrossRef]
19. Gao, B.; Zhu, H.; Liu, Z.; He, X.; Sun, J.; Li, Y.; Wu, X.; Pehrsson, P.; Zhang, Y.; Yao, Y.; et al. Chemical Compositions of Lianqiao (*Forsythia suspensa*) Extracts and Their Potential Health Benefits. *Pharmaceuticals* **2024**, *17*, 740. [CrossRef]
20. Li, J.; Qin, Q.; Zha, S.H.; Zhao, Q.S.; Li, H.; Liu, L.P.; Hou, S.B.; Zhao, B. Green extraction of forsythoside A, phillyrin and phillygenol from *Forsythia suspensa* leaves using a β-cyclodextrin-assisted method. *Molecules* **2022**, *27*, 7055. [CrossRef]
21. Huang, Y.; Cheng, N.; Zhi, Y.; Qiao, D.; Wang, Y.; Ma, M.; Ge, C.; Tao, W.; Liu, W. Phillyrin from *Forsythia suspensa* suppresses the proliferation, angiogenesis, and metastasis of colorectal cancer via targeting CD147. *J. Ethnopharmacol.* **2025**, *347*, 119759. [CrossRef]
22. Hu, N.; Wang, C.; Dai, X.; Zhou, M.; Gong, L.; Yu, L.; Peng, C.; Li, Y. Phillygenin inhibits LPS-induced activation and inflammation of LX2 cells by TLR4/MyD88/NF-κB signaling pathway. *J. Ethnopharmacol.* **2020**, *248*, 112361. [CrossRef]
23. Wang, F.; Cao, G.S.; Li, Y.; Xu, L.L.; Wang, Z.B.; Liu, Y.; Lu, J.Q.; Zhang, J.Y. Characterization of forsythoside A metabolites in rats by a combination of UHPLC-LTQ-Orbitrap mass spectrometer with multiple data processing techniques. *Biomed. Chromatogr.* **2018**, *32*, e4164. [CrossRef]
24. Ni, Y.; Zhuang, H.; Kokot, S. A high performance liquid chromatography and electrospray ionization mass spectrometry method for the analysis of the natural medicine, *Forsythia suspensa*. *Anal. Lett.* **2014**, *47*, 102–116. [CrossRef]
25. Ma, B.; Li, J.; Lou, T.; Liang, Y.; Wang, C.; Li, R.; Wang, T.; Liu, J.; Guo, Y.; Wang, Z.; et al. Comprehensive screening and identification of phillyrin metabolites in rats based on UHPLC-Q-Exactive mass spectrometry combined with multi-channel data mining. *Int. J. Anal. Chem.* **2020**, *2020*, 8274193. [CrossRef]
26. Xia, Y.; Yang, B.; Wang, Q.; Liang, J.; Wei, Y.; Yu, H.; Zhang, Q.; Kuang, H. Quantitative analysis and chromatographic fingerprinting for the quality evaluation of *Forsythia suspensa* extract by HPLC coupled with photodiode array detector. *J. Sep. Sci.* **2009**, *32*, 4113–4125. [CrossRef]

27. Shen, F.; Zou, L.S.; Wen, H.M.; Cui, X.B.; Yu, S.; Zhu, H.X.; Li, C.; Tian, G.; Shao, J.G. Qualitative evaluation of *Forsythia suspensa* by HPLC-PDA fingerprint combined with UFLC-Q-TOF-MS qualitative identification. *Zhongguo Zhong Yao Za Zhi* **2019**, *44*, 4495–4503.
28. Fu, K.; Ma, C.; Wang, C.; Zhou, H.; Gong, L.; Zhang, Y.; Li, Y. Forsythiaside A alleviated carbon tetrachloride-induced liver fibrosis by modulating gut microbiota composition to increase short-chain fatty acids and restoring bile acids metabolism disorder. *Biomed. Pharmacother.* **2022**, *151*, 113185. [CrossRef]
29. Ma, J.; Wang, Z.; Sun, Y.; Zheng, R.; Tan, H.; Zhang, H.; Jin, Z.; Wu, Y.; Sun, Z. Phillyrin: A potential therapeutic agent for osteoarthritis via modulation of NF-κB and Nrf2 signaling pathways. *Int. Immunopharmacol.* **2024**, *141*, 112960. [CrossRef]
30. Matos Leitão, M.; Euclides Silva-Filho, S.; Arena, A.C.; Heredia-Vieira, S.C.; Cardoso, C.A.L.; Kassuya, C.A.L. Antinociceptive and anti-inflammatory properties of aqueous extract obtained from *Serjania marginata* Casar leaves. *J. Ethnopharmacol.* **2023**, *304*, 116018. [CrossRef]
31. Youssef, F.S.; Ashour, M.L.; El-Beshbishy, H.A.; Ahmed Hamza, A.; Singab, A.N.B.; Wink, M. Pinoresinol-4-O-β-D-glucopyranoside: A lignan from prunes (*Prunus domestica*) attenuates oxidative stress, hyperglycaemia and hepatic toxicity in vitro and in vivo. *J. Pharm. Pharmacol.* **2020**, *72*, 1830–1839. [CrossRef]
32. Qu, Q.; Li, Y.; Dong, Q.; Li, S.; Du, H.; Wang, Z.; Gong, X.; Zhang, W.; Lv, W.; Chao, L.; et al. Comparative evaluation of *Forsythiae Fructus* from different harvest seasons and regions by HPLC/NIR analysis and anti-inflammatory and antioxidant assays. *Front. Pharmacol.* **2021**, *12*, 737576. [CrossRef]
33. Zhou, S.; Wu, L.; Zhang, Y.; Guo, Y.; Xi, J.; Li, D.; Ji, J. Variations in flavonoid metabolites among *Forsythia suspensa* populations in response to environmental heterogeneity. *Plants* **2025**, *14*, 3329. [CrossRef]

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