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Chemical Fingerprint Analysis and Quantitative Analysis of *Rosa Rugosa* by UPLC-DAD

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Abstract: A method based on ultra performance liquid chromatography with diode array detector (UPLC-DAD) was developed for quantitative analysis of five active compounds and chemical fingerprint analysis of *Rosa rugosa.* Ten batches of *Rosa rugosa* collected from different plantations in the Xinjiang region of China were used to establish the fingerprint. The feasibility and advantages of the used UPLC fingerprint were verified for its similarity evaluation by systematically comparing chromatograms with professional analytical software recommended by State Food and Drug Administration (SFDA) of China. In quantitative analysis, the five compounds showed good regression (R^2=0.999 5) within the test ranges and the recovery of the method was in the range of 94.2–103.8%. The similarities of the fingerprints of 10 batches of the samples were more than 0.981. The developed UPLC fingerprint method is simple, reliable and validated for the quality control and identification of *Rosa rugosa.* Additionally, simultaneous quantification of five major bioactive ingredients in the *Rosa rugosa* samples was conducted to interpret the consistency of the quality test. The results indicated that the UPLC fingerprint as a characteristic distinguishing method combining similarity evaluation and quantification analysis, can be successfully used to assess the quality and to identify the authenticity of *Rosa rugosa.*

Keywords: *Rosa rugosa*; UPLC-DAD; quality control; fingerprint

1. Introduction

Traditional Chinese medicine (TCM) has recently become an attractive subject for many scientists and drug producers. Many TCM, across history and cultures, have been used for medicinal purposes as alternative therapies based on plants in order to avoid drug adverse effects, and over the past years, many articles were reported. When TCM were used, particular attention must be also paid to standardization process. Additionally, the Food and Drug Administration (FDA) specifies certain labeling requirements for foods, supplements, and drugs, and the European Union requires that standardized herbal substances are reported as content of constituents with known therapeutic activity.

The genus *Rosa rugosa* Thunb. (Family Rosaceae) is conventionally used as medicinal plants in TCM. Rosa rugosa is distributed throughout the temperate regions of eastern Asia, including China, Japan and Korea. In Asia, it is a traditional herbal medicine for treating stomach ache, diarrhea, menoxenia, pain and chronic inflammatory disease [1]. Phytochemical studies conducted so far showed the isolation of tannins, flavonoids, terpenoids, triterpenoids, steroids, tocopheral and carotene [2]. Tannins and flavonoids are of special interest related to their activities, such as antioxidative, antidiabetes and antinflammatory activity associated with global diseases including diabetes mellitus, pain and chronic inflammatory [3].

The qualitative and quantitative analysis of major components, and the analysis of chemical fingerprint, which has been introduced and accepted by State Food and Drug Administration (SFDA) of China (2000) (State Food and Drug Administration of China 2000). Different cultivation areas and climatic conditions may vary the chemical constituents of *Rosa rugosa* significantly. Since application is growing steadily, development of a suitable quality control method was urgently required. The official Chinese pharmacopoeia (China Pharmacopoeia Committee 2010) does not include the quality evaluation of *Rosa rugosa*.

Among the chromatographic fingerprinting applied to the authentication and qualitative evaluation of botanical products over the past decade, high performance liquid chromatography (HPLC) fingerprinting emerges as the most widely used method because of its convenience and efficiency [4-6]. However, the acquisition of a fingerprint and quantitative analysis by these methods was a tedious operation, as it generally needed about one or more hours for a single run. In recent years, UPLC is emerging as a viable technique for quantitative and chemical fingerprint analysis of natural product, and some reports have appeared in literature on its applications in the fingerprinting and quantitative analysis of Chinese herbal medicines [7-8]. The results obtained in these references demonstrated that UPLC was indeed a very powerful tool in chromatographic fingerprinting applications and quantitative analysis of the components in these herbal medicines.

To the best of our knowledge, there are no reports regarding the fingerprint analysis of *Rosa rugosa.* The objective of this study was to establish an effective UPLC fingerprint method for the identification and quality evaluation of *Rosa rugosa.* The chromatograms of the extracted samples from different *Rosa rugosa* plantations of Xinjiang were compared visually and analyses by similarity evaluation. Moreover, twenty-three components in ten batches of *Rosa rugosa* were simultaneously quantitated by UPLC method.

2. Results and Discussion

2.1. Optimization of Analysis Conditions

The first step in the quantitation of the five standards in *Rosa rugosa* (structures shown in the figure 1) is the full extraction from the materials. The preliminary tests made in this study indicated that the different extraction methods (ultrasonication, reflux, or Soxhlet) have great influence on the content of the five standards. The contents of the five compounds are higher when subjected to the ultrasonic extraction standards method compared with those subjected to other methods. In order to optimize the ultrasonic extraction conditions different extraction solvents as methanol, ethanol and different combinations of water- ethanol having ethanol concentration as: 20 %, 40 %, 60 % 80 % and 95 %, v/v. Extraction time was varied in each case as: 30, 40, 50 or 60 min. By comparing the sum numbers and areas of characteristic peaks in each chromatogram of a different factor, the optimal condition for extraction of *Rosa rugosa* was selected as 5.0g powder of each dried sample and it was extracted with 100 ml of 60 %(v/v) aqueous ethanol in an ultrasonic bath for 60 min.

2.2. Optimisation of UPLC chromatographic conditions

In order to obtain the most useful chemical information and best separation in the fingerprint chromatograms of *Rosa rugosa*, the column, the mobile phase compositions, gradient elution procedure, and detection wavelength were optimised. Two kinds of reverse-phase columns, BEH Shield C18 column (100 mm × 2.1 mm, 1.8μm) and BEH C18 column (100 mm × 2.1 mm, 1.8μm) were investigated, the BEH Shield C18 column was found to be more suitable and gave good peak separation and sharp peaks.

In order to enhance the resolution as well as to restrain the ionisation of target compounds, formic acid was added to the binary mixture of acetonitrile-water. To acquire better selectivity and higher efficiency, different concentrations of formic acid (0.05%, 0.1% and 0.2%) in the acetonitrile phase were also investigated. The resulted optimized mobile phase consisting of acetonitrile -0.1%

formic acid (pH 2.98, v/v) and 0.1% formic acid solution (pH 2.67, v/v) was chosen for the determination of *Rosa rugosa* with a large number of peaks on the chromatogram achieved within 71 min. More detectable peaks could be obtained and the baseline was well improved around 260 nm, and therefore, better results for 5 target compounds in *Rosa rugosa* and reference standards could be obtained. Hence characteristic chromatographic patterns were obtained by using 260 nm as the detection wavelength. Optimal UPLC condition used in this study was shown in UPLC condition Section.

2.3. Method validation of quantitative analysis

The method of quantitative analysis was validated in terms of linearity, limit of detection (LOD) and recovery test. Linear regression analysis for each compound was performed by plotting the peak area (y) against the concentrations (x, mg/ml) of the mixed standard solution which was expressed as follow: $y = 1.79 \times 10^8 x - 4.14 \times 10^4$, $R^2 = 0.9999$ (for GA, the linear range is 0.02– 0.1 mg/ml); $y = 4.58 \times 10^8 x - 2.03 \times 10^5$, $R^2 = 0.9999$ (for EA, the linear range is 0.02–0.2 mg/ml)); $y = 7.12 \times 10^5 x - 2.28 \times 10^2$, $R^2 = 0.9995$ (for hyperoside, the linear range is 0.05–0.3 mg/ml)); $y = 1.84 \times 10^7 x - 1.14 \times 10^3$, $R^2 = 0.9999$ (for astragalin, the linear range is 0.02–0.1 mg/ml)); $y = 1.28 \times 10^7 x - 9.20 \times 10^3$, $R^2 = 0.9999$ (for kaempferol-3-O-sophoroside) the linear range is 0.02– 0.1 mg/ml));

The LOD and LOQ under the present UPLC method were determined at signal-noise ratio (S/N) of 3 and 10 respectively. Standard solution containing five reference compounds was diluted to a series of appropriate concentrations with methanol. The diluted solutions were injected into UPLC for analysis. The LOD for GA, EA, hyperoside, astragalin and kaempferol-3-O-sophoroside were 0.04, 0.08, 0.5, 0.2, and 0.08 μg/ml, respectively, which indicated that the analytical method was acceptable with sufficient sensitivity.

Figure.1: The chemical structures of the investigated compounds

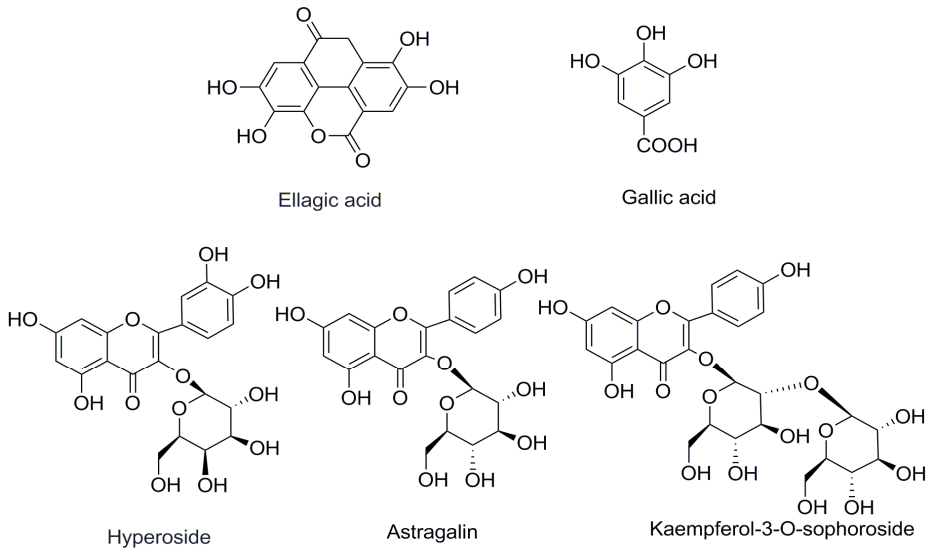
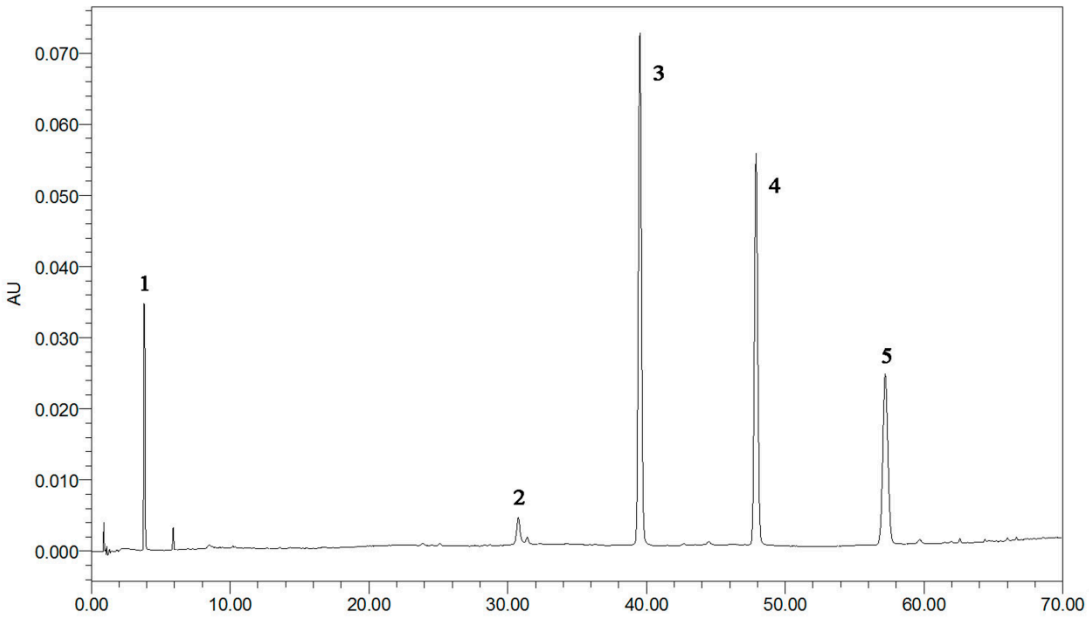


Figure.2 The typical UPLC chromatographic profile of five standards peaks 1, GA (3.8 3min); 2, EA (30.7 min); 3, Kaempferol-3-O-sophoroside (38.9 min) 4, Hyperoside (48.9 min); 5, Astragalin (57.2 min).



The recovery test was determined by standard addition method. The samples (Sample 1) were spiked with the high, intermediate and low levels of mixed five standards solution in triplicates, and then extracted, processed and quantified in accordance with the established procedures. The results of the recovery rates are summarized in Table 1. The recovery rates were performed using a Waters Acquity BEH Shield C18 column (100 mm 2.1 mm i.d., 1.8 μm), The recovery rates of the five compounds were in the range of 94.2–103.8% and their RSD values were less than 2.1%. Therefore, the UPLC-DAD methods were precise, accurate and sensitive enough for simultaneously quantitative evaluation of five compounds in *Rosa rugosa.*

Table 1: Recovery rates of the five components in *Rosa rugosa*

Component	Original(mg/g)	Added(mg/g)	Found(mg/g)	Recovery rate(%)	RSD(%)
Gallic acid	0.200	0.050	0.249	98.0%	1.6
	0.200	0.100	0.299	99.0%	1.8
	0.200	0.200	0.402	101.0%	1.0
Ellagic acid	0.460	0.120	0.573	94.2%	1.3
	0.460	0.230	0.683	97.0%	2.0
	0.460	0.460	0.904	96.5%	2.1
Hyperoside	118.870	29.720	148.030	98.1%	1.3
	118.870	59.430	177.370	98.4%	0.9
	118.870	118.870	236.910	99.3%	1.5
Astragalin	0.340	0.080	0.423	103.8%	0.9
	0.340	0.170	0.507	98.2%	2.1
	0.340	0.340	0.671	97.4%	1.7
Kaempferol-3-O-sophoroside l	0.120	0.030	0.149	96.7%	2.3
	0.120	0.060	0.178	96.7%	1.9
	0.120	0.120	0.234	95.0%	1.4

2.4. Method validation of UPLC fingerprint analysis

The method of UPLC fingerprint analysis was validated in terms of precision, repeatability and stability test. Intraday precision and repeatability as well as inter day stability of the UPLC fingerprint method were determined and expressed by the relative standard deviations (RSD) value of the average relative retention times (RRT) and relative peak areas (RPA) of the 23 characteristic common peaks with the reference peak (peak 8) at retention time (t_R) of 30.7min. By using the optimized conditions described above, the repeatability of the UPLC method was calculated by analysis five independently prepared solutions of the same sample (Sample 8). The variation of RRT and RPA of the characteristic peaks did not exceed 2.3% and 2.5%, respectively. The intraday precision variation of the RRT and RPA of the characteristic peaks was below 1% and 3%, respectively. This was obtained by analysis the five replicate sample solutions (Sample 8) continuously on the same day. The inter day stability test was assessed by analysis the same sample solution (Sample 8) on two consecutive days at different time intervals (0, 6, 12, 24, and 48 h) and the RSD values of RRT as well as RPA of the characteristic common peaks were less than 2.3% and 2.5%, respectively. The observed results indicated that the sample solution was stable within 48 h. The results of the precision, stability and repeatability tests are shown in Table 2 which met the national standard of traditional Chinese medicine fingerprint (SFDA, 2000).

Table 2: Analytical results of precision, stability and repeatability tests of 23 characteristic common peaks in *Rosa rugosa* samples (Sample 8) (n = 5).

Peak No.	RSD of RRT (%)			RSD of RPA (%)		
	Precision	Stability	Repeatability	Precision	Stability	Repeatability
1	0.11	0.14	0.18	0.16	0.09	0.12
2	0.24	0.19	0.17	0.21	0.13	0.17
3	0.22	0.08	0.04	0.11	0.15	0.21
4	0.13	0.09	0.15	0.18	0.18	0.27
5	0.17	0.2	0.17	0.19	0.21	0.19
6	0.12	0.29	0.2	0.11	0.22	0.21
7	0.09	0.25	0.19	0.19	0.16	0.14
8(S)	0	0	0	0	0	0
9	0.08	0.21	0.22	0.11	0.19	0.18
10	0.11	0.22	0.13	0.12	0.08	0.26
11	0.19	0.13	15	0.13	0.23	0.25
12	0.16	0.14	0.27	0.21	0.12	0.16
13	0.17	0.19	0.18	0.19	0.22	0.26
14	0.2	0.17	0.17	0.09	0.21	0.17
15	0.12	0.14	0.22	0.06	0.2	0.26
16	0.18	0.23	0.11	0.16	0.11	0.19
17	0.19	0.15	0.16	0.24	0.25	0.15
18	0.15	0.2	0.1	0.16	0.07	0.14
19	0.12	0.17	0.11	0.18	0.16	0.18
20	0.13	0.14	0.26	0.11	0.17	0.19
21	0.1	0.29	0.11	0.23	0.14	0.14
22	0.23	0.26	0.19	0.21	0.15	0.15
23	0.25	0.27	0.23	0.21	0.16	0.17

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Figure.3: The reference fingerprint of Rosa rugosa: (1) GA (3.783 min); (5) 2-Phenylethyl-O-β-D-glucopyranoside (26.9 min); (6) Quercetin -3-O-(2′′-O-β-D-glucopyranosyl)- β-D-glucopyranoside) (28.0min); (7) Juglanin (28.9 min) (8) EC (30.7 min); (9) Avicularin (36.0 min); (10) Quercetin (38.3 min); (11) Kaempferol-3-O-sophoroside (38.9 min) (16) Hyperoside (48.9 min); (18) Astragalin (57.3 min).

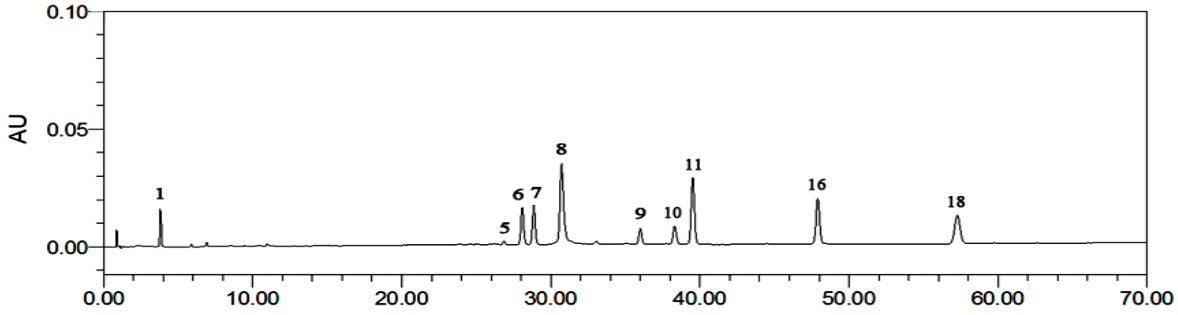
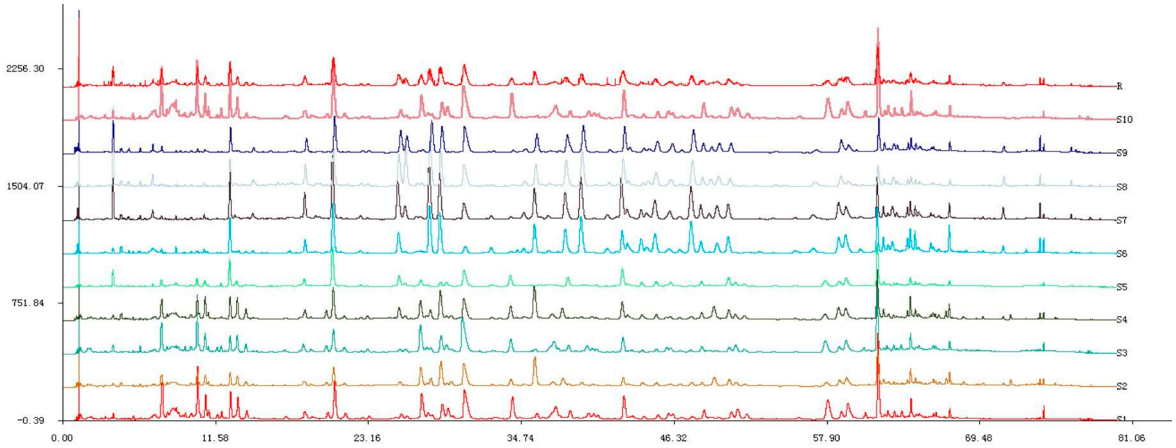


Figure.4: UPLC chromatographic fingerprints of 10 *Rosa rugosa* samples



To standardize the fingerprint, 10 samples were analyzed. The software named Similarity Evaluation System for Chromatographic Fingerprint of Traditional Chinese Medicine (Version 2004A) (Fig. 4) was used to evaluate chromatograms. These samples had similar UPLC profiles. Peaks that existed in all 10 samples with relatively high intensity and good resolutions were assigned as "characteristic common peaks" for identification of the plant. There were 23 characteristic peaks (from peak 1 to 23) found in the chromatogram, which covered more than 65% of the total area (Fig. 3). 10 components were identified as peak1 GA, peak5 2-Phenylethyl-O-β-D-glucopyranoside, peak6) Quercetin -3-O-(2′′-O-β-D-glucopyranosyl)- β-D-glucopyranoside), peak7 Juglanin (28.9 min) , peak8 EC (30.7 min), peak9 Avicularin (36.0 min), peak10 Quercetin (38.3 min) , peak11 Kaempferol-3-O-sophoroside (38.9 min) , peak16 Hyperoside (48.9 min), peak18 Astragalin (57.3 min).by comparing their retention time and UV spectrum with those of standard compounds. The other 13 common fingerprint peaks were unknown. To calculate the RRT and RPA of each characteristic peak, a reference peak should be chosen. Ellagic acid (peak 8) had a considerably high content of more than 0.46% of the total area, and it also had moderate retention time, stable peak area and good shape in the *Rosa rugosa* chromatograms. Therefore, it was chosen as the reference peak. Then the retention time and peak area of the 23 common peaks were measured; RRT and RPA of all characteristic common peaks with respect to this reference peak were calculated (Table 3).

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Table 3: The retention time (tR), relative retention time (RRT), peak area (PA) and relative peak area (RPA) of 23 common peaks in *Rosa rugosa* (n = 10).

	t_R	RRT		PA	RPA	
component	(min)	Average	RSD(%)	(mVs)	Average	RSD(%)
1	3.8	0.123	0.06	189231	0.026	0.17
2	12.6	0.412	0.11	1729467	0.236	0.10
3	18.3	0.597	0.19	909481	0.124	0.19
4	20.4	0.666	0.03	3648219	0.498	0.28
5	26.9	0.874	0.13	1834719	0.251	0.25
6	28.1	0.914	0.23	3984770	0.544	0.09
7	28.9	0.940	0.18	3443018	0.470	0.25
8	30.7	1.000	0.00	7320021	1.000	0.00
9	36.0	1.172	0.18	2647067	0.362	0.23
10	38.3	1.248	0.10	2577145	0.352	0.15
11	39.5	1.287	0.17	3672742	0.502	0.01
12	42.4	1.380	0.17	1878557	0.257	0.26
13	43.8	1.519	0.12	1391505	0.190	0.24
14	44.9	1.462	0.13	2255259	0.308	0.14
15	47.9	1.560	0.22	3443432	0.470	0.16
16	48.4	1.575	0.01	1227205	0.168	0.17
17	50.4	1.642	0.08	1738039	0.237	0.25
18	57.3	1.864	0.16	550902	0.075	0.26
19	58.8	1.915	0.19	1846625	0.252	0.19
20	59.3	1.932	0.07	2328296	0.318	0.11
21	61.8	2.011	0.21	2595214	0.355	0.19
22	64.6	2.104	0.14	984376	0.134	0.14
23	67.2	2.189	0.19	1214014	0.166	0.12

2.5. Similarity analysis of HPLC fingerprints of Rosa rugosa samples

The similarities between the entire chromatographic profiles of ten batches of *Rosa rugosa* and the standard chromatographic fingerprint were calculated by Similarity Evaluation System for Chromatographic Fingerprint of Traditional Chinese Medicine (Version 2004A), and the correlation coefficients of all 10 sample fingerprints were shown as 0.991, 0.995, 0.994, 0.998, 0.999, 0.981, 0.997, 0.998, 0.998, and 0.999. These results showed that the ten batches of *Rosa rugosa* from different plantations shared nearly the same correlation coefficients of similarities. In general, the common pattern of the ten batches test samples could be applied as a reference UPLC fingerprint to identify as well as to assess *Rosa rugosa.*

2.6. Quantitative determination of ten components in Rosa rugosa

In this study, the proposed UPLC method was successfully applied to the simultaneous determination of GA, EA, Kaempferol-3-O-sophoroside, Hyperoside, Astragalin in *Rosa rugosa* samples. The identity of the marker compound peaks in the chromatogram was confirmed by their retention times and their UV profiles. Quantification was based on the external standard method using calibration curves fitted by linear regression analysis. The contents of the five marker compounds in ten batches of the external standard method from different areas of *Rosa rugosa* are summarised in Table 4.

Contents of the Hyperoside 92.8, Astragalin 0.5 and GA 0.4 (mg/g) were significant and EA yielded the lowest amount 0.4 (mg/g) in this study while the amount of Kaempferol-3-O-sophoroside was 0.4 (mg/g). The %RSD are the results of the three replicate injections of the plant extracts. From

our literature review regarding the plant species under study, it appears that these five compounds have not been quantified before and are reported for the first time in this paper.

S1-5 of *Rosa rugosa* were collected from north of Xinjiang while the last parts were collected from south of Xinjiang. The Average contents of Hyperoside, Astragalin, Kaempferol-3-O-sophoroside were and GA in *Rosa rugosa* collected from south of Xinjiang higher than that in north of Xinjiang. The Average contents of EA higher than that in south of Xinjian (Table 4).

Table 4 Contents of ten components in *Rosa rugosa* (n = 3, mg/g).

sample No.	content of investigated components				
	Gallic acid	Ellagic acid	Hyperoside	Astragalin	Kaempferol
S1	0.20	0.46	118.87	0.34	0.12
S2	0.17	0.43	10.03	0.31	0.11
S3	0.37	0.30	46.76	0.47	0.10
S4	0.19	0.42	75.65	0.29	0.09
S5	0.19	0.28	55.16	0.51	0.05
Average(S1-5)	0.22	0.38	61.29	0.38	0.09
S6	0.43	0.32	82.76	0.53	0.05
S7	0.23	0.19	123.80	0.62	0.77
S8	0.79	0.49	203.40	1.12	1.20
S9	0.88	0.40	136.18	0.60	0.75
S10	0.37	0.31	75.65	0.44	0.48
Average(S6-10)	0.54	0.34	124.36	0.66	0.65
Average(S1-10)	0.38	0.36	92.83	0.52	0.37
RSD(%)	0.67	0.26	0.59	0.46	1.09

3. Materials and Methods

3.1. Materials

Ten batches of raw material samples of *Rosa rugosa* were collected from Xinjiang Uyghur Autonomous Region, China. S1 was collected from Qitai of Xinjiang. S2 – S5 were collected from Jimsar o f Xinjiang. S6 was collected from ShaChe of Xinjiang. S7 was collected from YuTan of Xinjiang. S8 was collected from HuTan of Xinjiang. S9 was collected from Hutan country of Xinjiang. S10 was collected from MoYu of Xinjiang.All the voucher specimens identified by research fellow Guanmian Shen, Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences.

3.2. Reagents

Acetonitrile (Fisher, optima®, LC-MS grade, Fair Lawn, NJ 07410, U.S.A.) and formic acid (Merck, EMSURE®, analytical grade, Darmstadt 64271, Germany) were used. Water used in the experiment was deionized and further purified by the Milli-Q Plus water purification system (Millipore Ltd., Bedford, MA, USA). Other reagents and chemicals were of analytical grade.

Standard preparation: The five chemical standards (hyperoside, gallic acid, ellagic acid; astragalin, Kaempferol-3-O-sophoroside were confirmed by UV, ESI-MS, and the chromatogram of mixture standards was shown in Figure 1. The purity of each compound was determined to be higher than 98% by normalization of the peak area detected by HPLC. The reference compounds were accurately weighed and dissolved in methanol and diluted to appropriate concentration ranges for the establishment of calibration curve. All stock and working standard solutions were stored at 4 ℃ until used for analysis.

Sample preparation: Dried and finely powdered flowers of *Rosa rugosa* were extracted with 60% aqueous ethanol (100 mL) for 1 hour under reflux. The solution was filtered through a 0.22 μm filter before UPLC analysis.

3.3. UPLC condition

UPLC analysis was performed on a Waters Acquity UPLC™ system (Waters, Milford, MA, USA) equipped with binary solvent delivery pump, an auto sampler and photodiode array detector (PAD). The instrument was controlled by Waters Empower 2 software. The chromatographic separation was performed using a Waters Acquity BEH Shield C18 column (100 mm 2.1 mm i.d., 1.8 μm, Waters, Massachusetts, U.S.A.), operated at 35 ℃. The mobile phase consisted of 0.1 % formic acid–acetonitrile (A) and 0.1 % formic acid–water (B) with a gradient elution of 2 % A (0–1 min), 2–5 % A (1–2 min), 5-10 % A (2–7 min), 10-11 % A (7–10 min), 11-18 % A (10–56 min), 18-28 % A (56-69 min), 28-43 % A(69–71min). Chromatograms were recorded at an absorbance of 260 nm. The mobile phase was eluted at a flow rate of 250 μL min-1, and injection volume was 2.00 μL.

Standard curves, limits of detection and recovery rates of quantitative analysis: The standard curves were obtained by plotting the peak area against nominal concentration of each compound and were fitted to a linear function of type y = ax + b. In this equation, y and x represent peak area and nominal concentration in mg/L, respectively. The limit of detection (LOD) was estimated as the minimum concentration of the compounds needed to produce signals that were at least three times stronger than the noise signal (S/N). The accuracy tests were carried out by spiking the known contents of mixed standard solution into the known concentration of *Rosa rugosa* samples, and the assessment was done by analyses the three different spiking concentrations of analyses in triplicates. The percent recovery rates for the analyses were presented as mean of the three results.

The precision of the UPLC fingerprint method was determined by analyses the replicated extraction solution of the same sample five times within a day. The sample stability test was determined with one sample on two consecutive days. The repeatability was assessed by analyses five independently prepared extraction solutions of *Rosa rugosa* samples. During this period, the solution was stored at room temperature.

Establishment of UPLC fingerprint and similarity analysis: To establish the representative chromatographic fingerprint, ten batches of *Rosa rugosa* samples were analyzed under the established UPLC method. The obtained UPLC data from ten batches of *Rosa rugosa* samples were exported from Waters Empower 2 software in AIA format and imported to the professional software named Similarity Evaluation System for Chromatographic Fingerprint of Traditional Chinese Medicine (Version 2004A). This system could reflect the similarity of the distribution ratio of the chemical composition accurately, as recommended by the SFDA.

5. Conclusions

In this study, UPLC-DAD method proved to be simple, accurate, and reliable for developed UPLC fingerprint and the determination of five bioactive compounds in *Rosa rugosa.* For the fingerprint analysis, 23 characteristic fingerprint peaks were applied to evaluate the similarities among ten batches of *Rosa rugosa* and they showed good similarities. For the quantitative determination, five components of ten batches of *Rosa rugosa* were successfully separated and determined. The UPLC fingerprint method was well validated by systematically comparing chromatograms of all samples from different regions. The method developed in this study will provide an important reference to establish the quality control method for other related traditional Chinese medicinal preparations.

Supplementary Materials: The following are available online at www.mdpi.com/link, Figure S1: title, Table S1: title, Video S1: title.

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

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Sample Availability: Samples of the compounds GA, EA hyperoside, astragalin and Kaempferol-3-O-sophoroside are available from the authors.