Study on the correlation between the fingerprint of Rosa rugosa and its formula granules

Yanyan Zhang1,a, Ping Qin1,b, Lijuan Lv1,c, Xiaosong Yang1,d, Hongmei Wu1,e, Juan kong1,f and Xiangpei Wang1,*

1School of Department of Pharmacy, Guizhou University of Traditional Chinese Medicine,Guiyang, Guizhou, China
2National Medical College, Guizhou Minzu University, Guiyang, Guizhou, China
3zhangyanyan0851@126.com, 41y69314111@qq.com, 5794453390@qq.com, 61692178417@qq.com, 7whm0425@126.com, 82898784668@qq.com

*Corresponding author’s e-mail: wxp0123@126.com

Abstract. As one of the medicinal and food homologous plants, Rosa rugosa has great development potential. Rosa rugosa formula granules has the features of easy to dispensing, carry, store and no decocting and so on. However, there is a lack of effective quality standards and quality monitoring system. Therefore, high performance liquid chromatography(HPLC) is used in this paper, taking the characteristic spectrum as the control, the correlation between the medicinal materials and the formula granules was evaluated. There were 8 common characteristic peaks in the characteristic spectrum of Rosa rugosa and formula granules, showing good correlation. Among the eight common peaks, rutin was identified. The fingerprint method established in this study has good precision, repeatability, stability, short analysis time and strong specificity, which can provide scientific basis for the quality evaluation and control of Rosa rugosa.

1. Introduction
Rosa rugosa is derived from the dried flower buds of the Rosa rugosa Thunb. It has the effect of promote qi flow to relieve depression, harmonize the blood and relieve pain[1]. It is used for the treatment of liver-stomach qi pain, low food intake and nausea, menstrual irregularities, traumatic injury[2]. The main components include volatile oil[3], flavonoids[4], tannin, sesquiterpenes, esters, vitamin, polysaccharide, amino acid, protein, alkaloids and so on[5-9]. Rose rugosa is one of 101 species of medicinal and edible homologous plants in China, and has great development potential[10]. Rose rugosa formula granules are fed with Rose rugosa medicine, and then through standard extraction, concentration, drying and granulation[11]. It has the features of easy to dispensing, carry, store and no decocting and so on. However, due to the lack of effective quality standards and quality monitoring system, there are some problems among these formulations, such as unclear quality standards, inconsistent doses and uneven quality[12]. In 2016, the State Pharmacopoeia Committee issued Technical requirements for quality control and standard formulation of traditional Chinese medicine dispensing granules (Draft), it is pointed out that the characteristics or fingerprints of traditional Chinese medicine and formula granules should be correlated[13]. At present, there are few studies on the correlation between HPLC fingerprint of Rose rugosa and formula granules. Therefore, the fingerprint method established in this study, in order to discuss the correlation between the fingerprint of rose rugosa and its formula granules, and to provide basis for quality control of the whole preparation process of rose rugosa formula granules.
2. Materials and instruments
Waters-2695 high performance liquid chromatography (American Waters Company), PDA detector, the number-display constant temperature water-bath HH-6 (Changzhou Aohua Instrument Co., Ltd), Al204-ic/One thousandth analytical balance (METTLE RTOLEDO Instruments Co., Ltd.), “Similarity Evaluation system of chromatographic fingerprint of traditional Chinese Medicine” (National Pharmacopoeia Commission, version 2012A), Ludin reference substance (a technology Co., Ltd., content ≥ 98%), methanol and phosphoric acid are analytical alcohols, acetonitrile as chromatographic purity and water as pure water. The source of rose rugosa and its formula granule is shown in Table 1.

Table 1. Sources of Rose rugosa and dispensing granules (S1-S10 are Rose rugosa, S11-S20 are dispensing granules).

| NO | source                                          | NO | source                                          |
|----|-------------------------------------------------|----|-------------------------------------------------|
| S1 | Anhui A Chinese herbal medicine market          | S11| Guangdong A Pharmaceutical Co., Ltd.           |
| S2 | Henan B Chinese herbal medicine market          | S12| Guangdong A Pharmaceutical Co., Ltd.           |
| S3 | Chengdu C Chinese herbal medicine market        | S13| Guangdong A Pharmaceutical Co., Ltd.           |
| S4 | Hebei D Chinese herbal medicine market          | S14| Guangdong A Pharmaceutical Co., Ltd.           |
| S5 | Jiangxi E Chinese herbal medicine market        | S15| Guangdong A Pharmaceutical Co., Ltd.           |
| S6 | Guangzhou F Chinese herbal medicine market      | S16| Guangdong A Pharmaceutical Co., Ltd.           |
| S7 | Shandong G Chinese herbal medicine market       | S17| Guangdong A Pharmaceutical Co., Ltd.           |
| S8 | Chongqing H Chinese herbal medicine market      | S18| Guangdong A Pharmaceutical Co., Ltd.           |
| S9 | Lanzhou I Chinese herbal medicine market        | S19| Guangdong A Pharmaceutical Co., Ltd.           |
| S10| Guiyang G Chinese herbal medicine market        | S20| Guangdong A Pharmaceutical Co., Ltd.           |

3. Preparation of solution

3.1. Preparation of sample solutions
0.5 g of Rose rugosa powder was weighed accurately and put into a 50 ml conical flask, adding methanol 20mL, ultrasonic extraction for 30 min, remove and cool off, then according to quality, make up the quality loss reduction with methanol, shake well, filtering. The filtrate was filtered by 0.22μm membrane for reserve.

0.6 g of rose rugosa dispensing granules powder was weighed accurately and put into a 50 ml conical flask, adding methanol 20mL, ultrasonic extraction for 30 min, remove and cool off, then according to quality, make up the quality loss reduction with methanol, shake well, filtering. The filtrate was filtered by 0.22μm membrane for reserve.

3.2. Preparation of reference solution
Took appropriate amount of rutin reference substance and weigh it accurately. The reference solution containing 20μg/ml rutin was prepared with methanol.

4. Chromatographic conditions
The chromatographic column was Dikma Odyssil C18 (250mm × 4.6mm, 5μm), the detection wavelength was set at 254nm, the column temperature was 35℃. The injection volume was 10 μL, and the flow rate was 1.0 ml / min. A binary gradi-ent elution system consisting of acetonitrile(A) and
water containing 0.1% phosphoric acid(B) was used with the following gradient programs, shown in Table 1.

Table 2. Mobile phase gradient elution conditions.

| Time (min) | Acetonitrile A (%) | 0.1% phosphoric acid water B (%) |
|-----------|-------------------|---------------------------------|
| 0~12      | 5~10              | 95~90                           |
| 12~60     | 10~21             | 90~79                           |
| 60~70     | 21~24             | 79~76                           |

5. Methodological study

5.1. Precision
Took the same Rose rugosa sample and prepare the test solution according to the method of preparation of sample solutions. The samples were injected 6 times continuously according to the chromatographic conditions, and the injection volume was 10μL, and to evaluate the precision of the instrument. The RSD of retention time and peak area of each common peak were lower than 3.0%, which indicated that the instrument had good precision.

5.2. Sample stability
Took the same Rose rugosa sample and prepare the test solution according to the method of preparation of sample solutions. The samples were injected at 0, 2, 4, 8, 12 and 24 hours according to the chromatographic conditions, the injection volume was 10μL, and to evaluate the stability of the samples. The RSD of relative retention time and relative peak area of each common peak were calculated to be lower than 3.0%, indicated that the sample solution remained stable for 24h.

5.3. Repeatability
Six samples of the same sample were taken, and the test solution samples were prepared in parallel according to the preparation method of preparation of sample solutions. The samples were injected according to the chromatographic conditions, the injection volume was 10μL, and to evaluate the repeatability of the experimental method. And RSD of the relative retention time and relative peak area of each common peak were calculated to be less than 3.0%, which showed that the method had good repeatability.

6. Establishment of HPLC fingerprint
10 batches of Rose rugosa and 10 batches of formula granule samples were taken for precise weighing, samples of test solution were prepared according to the preparation method of test solution, and they were injected and analyzed according to chromatographic conditions. The obtained chromatogram data files were imported into the software of "Similarity Evaluation System of Chromatographic Fingerprint of Traditional Chinese Medicine" for analysis, the HPLC fingerprints of Rose rugosa medicinal material and its formula particles were obtained. The results are shown in Figure 1, Figure 2.

7. Analysis of fingerprints

7.1. Similarity evaluation
The chromatographic data of Rose rugosa and its formula particle samples were imported into the software of "Similarity Evaluation System of Chromatographic Fingerprint of Traditional Chinese Medicine" for matching, the time window was 0.20. Consistency of chromatographic peak similarity was investigated by median method. The similarity between 10 batches of Rose rugosa medicinal
materials and 10 batches of formula granules was obtained. The similarity of medicinal materials and granules was evaluated respectively, the results are shown in Table 3. To evaluate the similarity of medicinal materials and formula granules, the results are shown in Table 4. S1-S10 are Rose rugosa, S11-S20 are dispensing granules.

7.2. Determination of common peaks
The fingerprints of Roses rugosa and their formula particles were analyzed, 10 batches of Rose rugosa were identified with 17 common chromatographic peaks, 10 batches of dispensing granules were identified with 14 common chromatographic peaks. There were 8 common peaks between Rose rugosa and formula granules, the results are shown in Figure 2, Figure 4 and Figure 6. Comparison with the reference spectra of rutin, could identify rutin chromatographic peak. Then using rutin as reference peak (S), the relative retention time and relative peak area of each common peak were calculated, as shown in Table 5, Table 6, Figure 1~Figure 6.

**Table 3.** Evaluation of the similarity of the fingerprint of the roses rugosa and roses rugosa dispensing granules.

| medicinal material | similarity | dispensing granules | similarity |
|-------------------|------------|---------------------|------------|
| S1                | 0.928      | S11                 | 1.000      |
| S2                | 0.978      | S12                 | 0.999      |
| S3                | 0.981      | S13                 | 1.000      |
| S4                | 0.941      | S14                 | 1.000      |
| S5                | 0.972      | S15                 | 1.000      |
| S6                | 0.981      | S16                 | 0.998      |
| S7                | 0.945      | S17                 | 1.000      |
| S8                | 0.947      | S18                 | 0.999      |
| S9                | 0.963      | S19                 | 0.997      |
| S10               | 0.978      | S20                 | 1.000      |

**Table 4.** Comparison results of similarity between roses rugosa and its formula granules.

| medicinal material | similarity | formula granules | similarity |
|-------------------|------------|------------------|------------|
| S1                | 0.859      | S11              | 0.992      |
| S2                | 0.881      | S12              | 0.991      |
| S3                | 0.895      | S13              | 0.993      |
| S4                | 0.892      | S14              | 0.993      |
| S5                | 0.910      | S15              | 0.993      |
| S6                | 0.902      | S16              | 0.990      |
| S7                | 0.886      | S17              | 0.992      |
| S8                | 0.886      | S18              | 0.990      |
| S9                | 0.899      | S19              | 0.997      |
| S10               | 0.889      | S20              | 0.993      |
Figure 1. HPLC fingerprint of Rose rugosa.

Figure 2. Control fingerprint of Rosa rugosa (peak 13 is rutin).

Figure 3. HPLC of Rose rugosa formula granules.
Figure 4. Control fingerprint of Rose rugosa formula granules (peak 13 is rutin).

Figure 5. Fingerprint of Rose rugosa and formula granules (S1-S10 is medicinal material; S11-S20 is formula granule).

Figure 6. Control fingerprint of Rose rugosa and formula granules (peak 8 is rutin).
### Table 5. Relative retention time of shared peaks.

| A Common peak | Common peaks of A and B |
|---------------|------------------------|
| S1            | -                      |
| S2            | 0.154 0.154 0.153 0.153 0.153 0.154 0.153 0.153 0.155 0.157 0.165 0.164 0.163 0.164 0.162 0.164 0.164 0.164 0.164 0.164 0.164 |
| S3            | 1                      |
| S4            | 0.184 0.181 0.181 0.181 0.180 0.180 0.180 0.181 0.181 0.181 0.181 |
| S5            | 0.220 0.217 0.216 0.216 0.217 0.215 0.216 0.216 0.216 0.216 0.215 |
| S6            | 0.220 0.217 0.216 0.216 0.217 0.215 0.216 0.216 0.216 0.216 0.215 |
| S7            | 0.220 0.217 0.216 0.216 0.217 0.215 0.216 0.216 0.216 0.216 0.215 |
| S8            | 0.220 0.217 0.216 0.216 0.217 0.215 0.216 0.216 0.216 0.216 0.215 |
| S9            | 0.220 0.217 0.216 0.216 0.217 0.215 0.216 0.216 0.216 0.216 0.215 |
| S10           | 0.220 0.217 0.216 0.216 0.217 0.215 0.216 0.216 0.216 0.216 0.215 |
| S11           | 0.220 0.217 0.216 0.216 0.217 0.215 0.216 0.216 0.216 0.216 0.215 |
| S12           | 0.220 0.217 0.216 0.216 0.217 0.215 0.216 0.216 0.216 0.216 0.215 |
| S13           | 0.220 0.217 0.216 0.216 0.217 0.215 0.216 0.216 0.216 0.216 0.215 |
| S14           | 0.220 0.217 0.216 0.216 0.217 0.215 0.216 0.216 0.216 0.216 0.215 |
| S15           | 0.220 0.217 0.216 0.216 0.217 0.215 0.216 0.216 0.216 0.216 0.215 |
| S16           | 0.220 0.217 0.216 0.216 0.217 0.215 0.216 0.216 0.216 0.216 0.215 |
| S17           | 0.220 0.217 0.216 0.216 0.217 0.215 0.216 0.216 0.216 0.216 0.215 |
| S18           | 0.220 0.217 0.216 0.216 0.217 0.215 0.216 0.216 0.216 0.216 0.215 |
| S19           | 0.220 0.217 0.216 0.216 0.217 0.215 0.216 0.216 0.216 0.216 0.215 |
| S20           | 0.220 0.217 0.216 0.216 0.217 0.215 0.216 0.216 0.216 0.216 0.215 |

### Table 6. Relative peak area of common peaks.

| A Common peak | B Common peak | Common peaks of A and B |
|---------------|---------------|------------------------|
| S1            | -             | 0.026 0.023 0.029 0.027 0.027 0.027 0.027 0.027 0.027 0.027 0.027 |
| S2            | 0.312 0.167 0.183 0.420 0.165 0.166 0.441 0.443 0.315 0.164 0.389 0.440 0.381 0.380 0.391 0.369 0.404 0.429 0.396 0.382 |
| S3            | -             | -                      |
| S4            | -             | -                      |
| S5            | -             | -                      |
| S6            | -             | -                      |
| S7            | -             | -                      |
| S8            | -             | -                      |
| S9            | -             | -                      |
| S10           | -             | -                      |
| S11           | -             | -                      |
| S12           | 0.023 0.024 0.023 0.023 0.023 0.022 0.023 0.020 0.020 0.020 0.020 0.020 0.020 0.020 0.020 0.020 0.020 0.020 0.020 0.020 0.020 0.020 |
| S13           | -             | -                      |
| S14           | -             | -                      |
| S15           | -             | -                      |
| S16           | -             | -                      |
| S17           | -             | -                      |
| S18           | -             | -                      |
| S19           | -             | -                      |
| S20           | -             | -                      |

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8. Discussion

HPLC fingerprint identification technology can show the homogeneity and stability of chemical constituents between Rose rugosa and its formula particles, it can also identify the similarity between the fingerprints of rose medicinal herbs and their formula particles and the difference in the number of common peaks. It reveals a series of preparation processes of roses from medicinal herbs to its formulated granules, and changes in chemical composition during preparation. The results showed that there were 10 batches of Rose rugosa were identified with 17 common chromatographic peaks, 10 batches of dispensing granules were identified with 14 common chromatographic peaks. There were 8 common peaks between Rose rugosa and formula granules, it is suggested that these eight characteristic components can be traced back in the preparation process of Rose rugosa formula granules. HPLC fingerprint identification technology was applied to the whole process monitoring of traditional Chinese medicine formula granules, it can reflect the change law of quantity and quality transfer in each link of production process. So as to provide reference for the quality control of stability and homogeneity of Rose rugosa formula granules preparation.

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