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Identification of flowers of *Musa nana* Lour., flowers of *Musa basjoo* Sieb. et Zucc and flowers of *Musa balbisiana* Colla by HPLC fingerprinting

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Abstract. Objective: HPLC fingerprint identification method of flowers of *Musa nana* Lour., flowers of *Musa basjoo* Sieb. et Zucc and flowers of *Musa balbisiana* Colla were established, and the chromatogram similarities difference of three kinds of flowers were compared, in order to provide reference for variety identification, resource development and utilization of flowers of *Musaceae* medicinal materials. Methods: The separation was performed on a Diamonsil C₁₈ column (4.6mm×250mm, 5μm) at 30°C. A binary gradient elution system was composed of acetonitrile and water containing 0.05% phosphoric acid and the flow rate was 0.8mL/min. The detection wavelength was 280nm. The HPLC fingerprint of 17 batches of flowers of *Musaceae* medicinal materials were determined and the similarities were evaluated. Results: The experimental results showed that the samples from flowers of same varieties showed high similarities, and had more common peaks. Flowers of *Musa nana* Lour., are compared with flowers of *Musa basjoo* Sieb. et Zuccr and flowers of *Musa balbisiana* Colla, whose similarities were less than 0.430 with few common peaks. However, the comparison between flowers of *Musa basjoo* Sieb. et Zuccr and flowers of *Musa balbisiana* Colla showed high similarities with more common peaks. The similarities of 17 batches of flowers of *Musaceae* medicinal materials were between 0.187 and 0.984 with few common peaks, indicating the significant difference of three kinds of flowers. Conclusion: The proposed method is simple, highly reproducible and suitable for the identification of flowers of *Musaceae* medicinal materials.

1. Introduction

Flowers of *Musa nana* Lour., flowers of *Musa basjoo* Sieb. et Zucc and flowers of *Musa balbisiana* Colla are the dried flower buds or flowers of *Musa nana* Lour., *Musa basjoo* sieb. et Zucc and *Musa balbisiana* Colla, respectively. Flowers of *Musa nana* Lour. are the wastes of banana after they are ripe and picked. It is eaten as a daily vegetable in Southeast Asia and Southwest China. It is rich in potassium and a variety of unsaturated fatty acids which can prevent hypertension and cardiovascular diseases[1-4]. Recently, studies have indicated that flowers of *Musa nana* Lour. extracts exhibited hypoglycemic, antioxidation and free radical scavenging activity [5-9]. The flowers of *Musa basjoo* Sieb. et Zucc are valuable herbs to eliminate sputum soft hard mass, calm the liver and promote blood circulation to remove stasis and possess various pharmacological actions, such as inhibitory activities against α -glucosidase, bacteriostasis in vitro and antioxidant activity[10-11]. Petroleum ether, ethyl acetate parts of flowers of *Musa basjoo* Sieb. et Zucc had inhibitory effect on the hepatoma BEL-7402



cells[12].

Owing to these benefits, many people use flowers of *Musa nana* Lour., flowers of *Musa basjoo* Sieb. et Zucc immersed in water to treat or prevent diabetes and cardiovascular disease. However, flowers of *Musa nana* Lour., flowers of *Musa basjoo* Sieb. et Zucc and flowers of *Musa balbisiana* Colla are easy confused edible in China. In addition, the circulation and use of the three kinds of flowers are confused also. At present, only simple properties and characteristics were used to identify three kinds of flowers. However, it is difficult to distinguish them because they are plants of the same family and genus, and are closely related to each other, and their medicinal materials are similar in character and microscopic characteristics. In addition, it is not reported whether the three kinds of flowers have the same effect and can replace each other. In order to control the quality of flowers of *Musaceae* medicinal materials comprehensively. HPLC fingerprint of flowers of *Musa nana* Lour., flowers of *Musa basjoo* Sieb. et Zucc and flowers of *Musa balbisiana* Colla were established in this study, and the results to comprehensively reflect the types and quantities of chemical components contained in the three flowers. It provides reference for the identification, accurate use and development and utilization of flowers of *Musaceae* medicinal materials.

2. Experimental

2.1 Sample Collection and Preparation

Flowers of *Musa nana* Lour., flowers of *Musa basjoo* Sieb. et Zucc and flowers of *Musa balbisiana* Colla has been identified as dried flower buds or flowers of *Musa nana* Lour., *Musa basjoo* Sieb. et Zucc, *Musa balbisiana* Colla by Professor Xiangpei Wang of Guiyang University of Chinese Medicine, respectively. The specific sources are shown in table 1. Plants were air-dried, crushed into fine powder, accurately weighed 1g, placed into round bottom flask, respectively. Reflux extraction samples twice times with 50mL Methanol, each time is 1.5h, combined with filtrate and water bath wipe dry. The residue is dissolved with methanol and its volume is 10mL. The residue is filtered by microporous membrane (0.22 micron) and the filtrate is obtained.

2.2 Chemicals and Reagents

HPLC-grade methanol and acetonitrile were obtained from Tianjin Kemiou Chemical Reagent Co., Ltd; the rest are analytical pure and the water is purchased from Hangzhou Wahaha Group Co., Ltd.

2.3 Instrumentation and Apparatus

HPLC(Waters e-2695) with PDA detector; HS-10260T ultrasonic cleaner (Tianjin Heng'ao Science and Technology Development Co., Ltd.); HH-6 Digital Display Constant Temperature Water Bath pot (Changzhou Aohua Instruments Co., Ltd.); AL204-IC/10000 analytical balance (Metler Instruments Co., Ltd.); Chinese Herbal Chromatographic Similarity Evaluation System(2004 A edition).

2.4 Chromatographic Conditions

The column was Diamonsil C₁₈ (4.6mm×250mm, 5μm). A binary gradient elution system consisting of acetonitrile(B) and water containing 0.05% phosphoric acid(A) was used with the following gradient programs: 0-15min, 2%-10%B; 15-35min, 10%-20%B; 35-50min, 20%-30%B; 50-65min, 30%-55%B; 65-80min, 55%-72%B; 80-85min, 72%-80%B; 85-110min, 80%-100%B; 110-120min, 100%-2%B, detection wavelength was set at 280nm, and the flow rate was 0.8mL/min. The injection volume was 20μL and the column temperature was maintained at 30℃.

2.5 HPLC method validation

2.5.1 Precision test. Samples of the same sample solution (Yunnan Jinghong 2) were injected six times in accordance with the chromatographic conditions under "2.4". The RSD of relative retention time

and relative peak area of each common peak were 0.01%-0.11% and 0.47%-2.26% , respectively; indicating that the instrument had good precision.

2.5.2 Stability test. The same sample solution (Yunnan Jinghong 2) was determined at 0, 2, 4, 8, 24 and 48h, respectively. The RSD of the relative retention time and relative peak area of the common peaks were 0.01%-0.114% and 0.10%-2.87%, respectively; it shows that the sample solution was stable within 48h.

2.5.3 Repeatability test. Six samples of Jinghong No. 2 in Yunnan Province were taken and the sample solution was prepared under "2.1" and determined under "2.4" chromatographic conditions. The RSD of relative retention time and relative peak area of each common peak ranged from 0.01% to 0.28% and from 0.23% to 2.02% , respectively; which showed that the method had good repeatability.

3. Data analysis

3.1 Establishment of HPLC fingerprint

A total of 17 batches of flowers of *Musa nana* Lour., flowers of *Musa basjoo* Sieb. et Zucc and flowers of *Musa balbisiana* Colla were taken and accurately weighed. The sample solution was prepared under "2.1" and analyzed under "2.4" chromatographic conditions. Samples were used to detect and analyze by HPLC-PDA chromatogram. The peak value, peak number and peak position of HPLC-PDA chromatogram were compared and analyzed the fingerprint of flowers of *Musaceae* medicinal materials. The relative retention time and area of the common peak of flowers of *Musaceae* medicinal materials were calculated. The results are shown in figure 1.

3.2 Establishment of Common Fingerprint Peaks

The 11 peaks with retention time of about 26.479 min were used as reference peaks S, and 22 common peaks were established. The relative retention time and relative peak area of each peak were calculated, as shown in table2-table3.

3.3 Similarity Evaluation of Fingerprint

The chromatographic data of 17 batches of flowers of *Musa nana* Lour., flowers of *Musa basjoo* Sieb. et Zuccr and flowers of *Musa balbisiana* Colla were imported into the "Chinese medicine chromatographic fingerprint similarity evaluation system 2004A edition" for matching. The time window was 0.20. The consistency of chromatographic peak similarity was examined by median method. The results are shown in table 1.

Table 1. Source of medicinal materials.

No.	species	producing area	Similarity
S1	flowers of <i>Musa basjoo</i> Sieb. et Zucc	Jinghong City, Yunnan Province	0.966
S2	flowers of <i>Musa basjoo</i> Sieb. et Zucc	Jinghong City, Yunnan Province	0.984
S3	flowers of <i>Musa basjoo</i> Sieb. et Zucc	Jinghong City, Yunnan Province	0.975
S4	flowers of <i>Musa basjoo</i> Sieb. et Zucc	Jinghong City, Yunnan Province	0.952
S5	flowers of <i>Musa basjoo</i> Sieb. et Zucc	Jinghong City, Yunnan Province	0.973
S6	flowers of <i>Musa basjoo</i> Sieb. et Zucc	Jinghong City, Yunnan Province	0.975
S7	flowers of <i>Musa basjoo</i> Sieb. et Zucc	Jinghong City, Yunnan Province	0.937
S8	flowers of <i>Musa balbisiana</i> Colla	Panyu City, Guangdong Province	0.864
S9	flowers of <i>Musa balbisiana</i> Colla	Panyu City, Guangdong Province	0.813
S10	flowers of <i>Musa balbisiana</i> Colla	Zhaoqing City, Guangdong Province	0.866

S11	flowers of <i>Musa balbisiana</i> Colla	Zhaoqing City, Guangdong Province	0.746
S12	flowers of <i>Musa balbisiana</i> Colla	Yulin City, Guangxi Province	0.749
S13	flowers of <i>Musa balbisiana</i> Colla	Yulin City, Guangxi Province	0.883
S14	flowers of <i>Musa nana</i> Lour.	Zhangzhou City, Fujian Province	0.187
S15	flowers of <i>Musa nana</i> Lour.	Zhangzhou City, Fujian Province	0.202
S16	flowers of <i>Musa nana</i> Lour.	Zhangzhou City, Fujian Province	0.370
S17	flowers of <i>Musa nana</i> Lour.	Zhangzhou City, Fujian Province	0.416

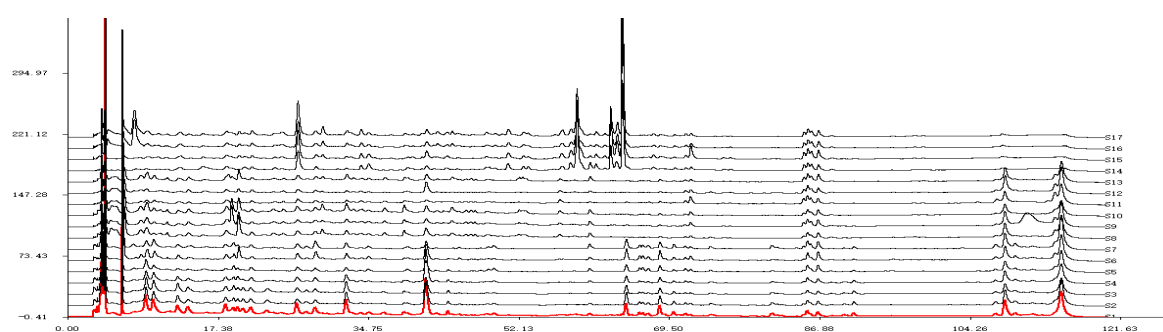


Figure 1. HPLC chromatogram of 17 batches of flowers of *Musa nana* Lour., flowers of *Musa basjoo* Sieb. et Zucc and flowers of *Musa balbisiana* Colla (S1-S7: flowers of *Musa basjoo* Sieb. et Zucc; S8-S13: flowers of *Musa balbisiana* Colla; S14-S17: flowers of *Musa nana* Lour).

Table 2. Relative retention time of HPLC fingerprints of flowers of *Musa nana* Lour., flowers of *Musa basjoo* Sieb. et Zucc and flowers of *Musa balbisiana* Colla.

Com mon peak	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15	S16	S17
1	0.146	0.146	0.142	0.146	0.146	0.146	0.144	0.147	0.147	0.147	0.147	0.147	0.147	0.147	0.147	0.147	0.147
2	0.155	0.155	0.146	0.155	0.155	0.155	0.148	0.156	0.156	0.156	0.156	0.156	0.155	0.156	0.155	0.156	0.156
3	0.164	0.164	0.155	0.164	0.163	0.164	0.157	0.164	0.164	0.164	0.163	0.164	0.163	0.163	0.163	0.163	0.163
4	0.235	0.235	0.235	0.235	0.235	0.236	0.241	0.237	0.238	0.237	0.237	0.237	0.237	0.238	0.237	0.237	0.237
5	0.341	0.341	0.342	0.341	0.341	0.342	0.347	0.344	0.345	0.346	0.344	0.344	0.346	0.338	0.346	0.346	0.345
6	0.374	0.374	0.374	0.374	0.374	0.374	0.378	—	—	—	—	—	—	—	—	—	—
7	0.689	0.688	0.689	0.688	0.688	0.688	0.690	0.689	0.691	0.692	0.689	0.688	0.698	0.690	0.689	0.690	0.690
8	0.723	0.723	0.723	0.722	0.722	0.723	0.724	0.743	0.743	0.744	0.742	0.742	0.743	—	—	—	—
9	0.742	0.741	0.741	0.739	0.739	0.740	0.744	—	—	—	—	—	—	—	—	—	—
10	0.800	0.798	0.797	0.798	0.798	0.798	0.801	0.801	0.800	0.802	0.800	0.799	0.800	0.800	0.800	0.800	0.801
11(S)	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
12	1.080	1.080	1.079	1.078	1.078	1.079	1.080	1.078	1.079	1.080	1.077	1.077	1.079	1.078	1.080	1.078	1.077
13	1.216	1.214	1.213	1.211	1.211	1.213	1.214	1.213	1.213	1.215	1.212	1.212	1.213	1.212	1.212	1.213	1.214
14	1.565	1.562	1.562	1.559	1.559	1.561	1.561	1.560	1.561	1.563	1.560	1.558	1.560	1.559	1.560	1.559	1.560
15	2.439	2.437	2.436	2.433	2.433	2.435	2.434	2.434	2.434	2.438	2.431	2.433	2.434	—	—	—	—
16	2.586	2.583	2.582	2.580	2.580	2.581	2.580	2.578	2.578	2.583	2.576	2.576	2.575	2.576	2.576	2.575	2.576
17	3.232	3.229	3.227	3.224	3.224	3.209	3.224	3.223	3.223	3.228	3.217	3.219	3.219	3.219	3.220	3.218	3.219

18	3.278	3.275	3.273	3.270	3.270	3.226	3.270	3.269	3.269	3.274	3.263	3.265	3.265	3.265	3.266	3.264	3.265
19	4.056	4.052	4.050	4.046	4.046	4.049	4.046	4.044	4.047	4.051	4.043	4.039	4.042	—	—	—	—
20	4.098	4.093	4.091	4.087	4.087	4.090	4.087	4.084	4.085	4.091	4.078	4.079	4.079	4.063	4.066	4.062	4.064
21	4.143	4.137	4.134	4.130	4.130	4.134	4.131	—	—	—	—	—	—	—	—	—	—
22	4.343	4.338	4.336	4.331	4.331	4.334	4.331	4.330	4.331	4.337	4.323	4.324	4.324	—	—	—	—

Table 3. Relative peak area of HPLC fingerprints of flowers of *Musa nana* Lour., flowers of *Musa basjoo* Sieb. et Zucc and flowers of *Musa balbisiana* Colla.

Comm on peak	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15	S16	S17
1	2.013	3.190	0.770	3.070	2.692	2.610	0.265	2.294	2.310	1.374	1.671	2.649	1.665	0.316	0.215	0.295	0.315
2	1.837	2.763	2.617	3.006	1.925	2.595	1.490	1.236	1.029	1.272	1.018	1.235	1.201	0.079	0.063	0.117	0.114
3	4.809	6.932	2.864	7.450	5.568	6.498	2.180	24.065	23.127	22.973	7.380	7.771	18.015	0.541	1.441	1.838	1.640
4	1.710	2.431	2.381	2.892	2.046	2.629	2.561	11.909	11.114	10.875	4.847	6.952	7.165	0.113	0.292	0.613	0.548
5	2.001	2.791	2.553	1.816	1.797	1.483	1.450	2.423	2.667	2.337	0.776	0.919	1.762	0.020	0.115	0.161	0.167
6	1.436	2.030	2.057	1.836	0.603	1.771	0.946	—	—	—	—	—	—	—	—	—	—
7	0.886	1.008	0.988	0.418	0.418	0.712	0.679	1.094	2.071	1.566	1.313	0.826	1.703	0.134	0.071	0.109	0.131
8	0.690	0.979	1.043	0.888	0.888	1.045	0.612	3.538	2.885	2.350	0.484	0.771	1.628	—	—	—	—
9	0.536	0.641	0.632	0.435	0.435	0.436	1.254	—	—	—	—	—	—	—	—	—	—
10	0.702	0.847	0.800	0.737	0.737	0.657	0.345	0.793	0.637	0.567	0.506	0.706	0.460	0.082	0.060	0.154	0.137
11(S)	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
12	0.717	0.802	0.737	0.648	0.648	1.527	1.186	0.775	0.825	0.843	0.631	0.768	0.664	0.140	0.141	0.179	0.145
13	1.175	1.716	1.630	0.589	0.589	0.898	0.864	0.954	1.070	1.292	0.467	0.569	1.129	0.081	0.071	0.146	0.147
14	3.728	3.998	2.842	2.245	2.245	0.842	3.218	2.160	1.445	1.093	0.426	3.423	1.411	0.199	0.275	0.244	0.182
15	0.898	1.612	1.170	1.070	1.070	1.395	1.391	0.304	0.280	0.170	0.213	0.210	0.253	—	—	—	—
16	0.848	1.292	0.693	0.999	0.999	0.818	1.326	0.218	0.297	0.223	0.300	0.255	0.304	0.062	0.024	0.031	0.021
17	0.243	0.368	0.288	0.577	0.577	0.440	0.595	1.124	1.843	0.902	1.692	1.901	1.466	0.310	0.322	0.278	0.207
18	0.364	0.572	0.416	1.204	1.204	1.291	1.257	1.150	1.898	0.984	1.269	1.508	1.661	0.269	0.319	0.259	0.190
19	0.266	0.593	0.288	0.299	0.299	0.846	0.389	0.390	0.522	0.171	1.119	0.579	0.391	—	—	—	—
20	1.642	2.865	1.931	2.188	2.188	3.371	2.503	4.079	5.348	2.124	3.848	6.656	3.915	0.036	0.027	0.031	0.022
21	0.209	0.564	0.400	0.378	0.378	0.743	0.394	—	—	—	—	—	—	—	—	—	—
22	0.516	1.001	0.705	1.245	1.245	1.865	1.045	8.099	9.640	4.344	7.644	10.858	6.341	—	—	—	—

4. Results and Discussion

The effects of different factors, such as 50% methanol, ethanol, 50% ethanol, different extraction time, reflux and ultrasound on the extraction of medicinal materials were examined. The results exhibited that the sample extracted by methanol reflux for 1.5h had more chromatographic peaks and complete information, and the method was stable with good repeatability. In the process of optimizing chromatographic conditions, various mobile phase systems were investigated, the results demonstrated that the mobile phase was acetonitrile and water containing 0.05% phosphoric acid, the separation degree of each peak was good and the baseline was stable, which was suitable for the analysis of the fingerprint of HPLC. In the investigation of wavelength, column temperature and flow velocity, it was found that at the wavelength of 280nm and the velocity of 0.8mL/min, information was rich in chromatogram, characteristic peak was significantly and the separation degree of each peak was good.

The experimental results showed that the similarities of 4 batches of flowers of *Musa nana* Lour. ranged from 0.877 to 0.987 with 19 common peaks. The similarities of 7 batches of flowers of *Musa basjoo* Sieb. et Zucc were between 0.937 and 0.984 with 22 common peaks, and the similarities of 6 batches of flowers of *Musa balbisiana* Colla ranged from 0.910 to 0.980 with 21 common peaks. Comparison of three varieties showed that the similarities between flowers of *Musa nana* Lour. and flowers of *Musa basjoo* Sieb. et Zucc were less than 0.420 with 15 common peaks, the similarities between flowers of *Musa nana* Lour. and flowers of *Musa balbisiana* Colla were less than 0.430 with 16 common peaks. And the similarities between flowers of *Musa balbisiana* Colla and flowers of *Musa basjoo* Sieb. et Zucc were less than 0.900 with 19 common peaks. The similarities of 17 batches of flowers of *Musaceae* medicinal materials were between 0.187 and 0.984. The results showed that high similarities of flowers of same variety, but the similarities of different varieties flowers were low, and the chemical composition were different. The proposed method is simple, highly reproducible and suitable for the identification of flowers of *Musaceae* medicinal materials. In addition, whether the three kinds of flowers of *Musaceae* medicinal materials can be used as the same medicinal material or not, it should be further studied on the difference of pharmacodynamic effects in combination with their functional treatment, so as to provide reference for the rational development and utilization and clinical application of flowers of *Musaceae* medicinal materials.

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