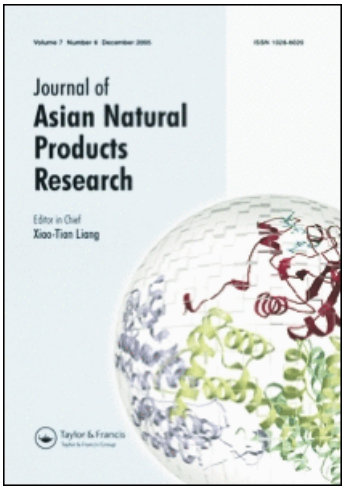


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# HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC FINGERPRINT EVALUATION OF THE QUINOLIZIDINE ALKALOIDS FROM COMMERCIAL RADIX SOPHORAE FLAVESCENTIS

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Fifteen commercial samples of Radix Sophorae Flavescentis were collected from different parts of China. HPLC analysis showed that the commercial samples all contained quinolizidine alkaloids and a total of nine chromatographic peaks were identified by referring to standard compounds, HPLC/MS analysis and comparison of the physicochemical data of the isolated peak with the literature. The contents of the major alkaloids were determined and the ratio of the major alkaloids contents was shown to be correlated with their source of origin. The commercial samples from China gave a distinct HPLC pattern showing the main alkaloids contents. The sample preparations were researched and the extraction conditions of the alkaloids were optimized. Reproducible HPLC fingerprints can be obtained for the quinolizidine alkaloids under the well-controlled extraction conditions. The HPLC fingerprint analysis method is suitable for the quality control of the Radix Sophorae Flavescentis and the standardization of phytomedicines.

*Keywords*: HPLC; Fingerprint analysis; Radix Sophorae Flavescentis; *Sophora flavescens*; Quinolizidine alkaloids

## INTRODUCTION

The discrepancy between the theory and practice of traditional Chinese medicine (TCM) requires some comprehensive evaluation measure to assess the quality of a Chinese herbal medication. This could be provided by analytical chemistry methods, from "decompose", microanalysis, towards to "macro", synthetic analysis. Chromatographic fingerprints or chromatographic image analysis is one specific approach to a more effective comparative and relative quality assessment of Chinese herbal medication [1].

The Radix Sophorae Flavescentis (RSF), one of the most important traditional Chinese herbal medicines, has been used as an antipyretic and diuretic agent since ancient times. In the Chinese Pharmacopoeia, RSF is listed as the roots of *Sophora flavescens* Ait [2].

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Early chemical studies on *Sophora flavescens* revealed that quinolizidine alkaloids were the main chemical components and they are also the characteristic constituents [3–5]. Up to now about twenty kinds of alkaloids have been isolated. The structures of nine major alkaloids such as sophoranol (**1**), oxymatrine (**2**), oxysophocarpine (**3**), *N*-methylcytisine (**4**), anagyrine (**5**), sophocarpine (**6**), isomatrine (**7**), matrine (**8**) and sophoridine (**9**) are listed in Fig. 1. HPLC analyses of these alkaloids have been reported elsewhere: under uncontrolled conditions it has not been possible to obtain satisfactory selectivity or reproducibility [6–8]. This paper reports an approach that gives an acceptable chromatogram for the separation of the main quinolizidine alkaloids. The HPLC fingerprint chromatograms are suitable for quantitative analysis and can be used as an effective tool for to evaluate commercial herbal medicines.

The commercial products on the market are quite complicated with respect to their origins. Thus a systematic comparative study of commercial samples is of importance. In this study, fifteen commercial samples were collected from the markets of China. Up to now, there have been no published general quality standards for RSF, although laboratories in various



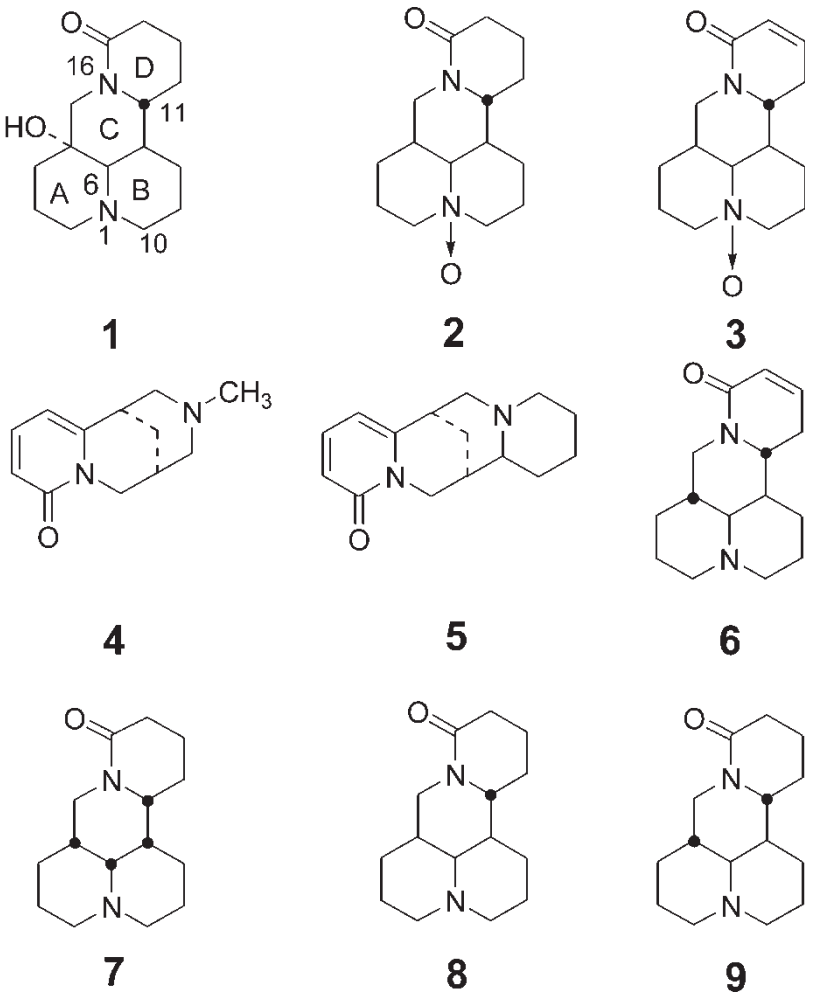


FIGURE 1 Structures of alkaloids **1**–**9**.

universities are engaged in the analysis of *Sophora flavescens* constituents. It is necessary that the standardization be established for the quality control of RSF phytomedicines.

Experimental work has shown the importance of the purification of the samples in improving the chromatographic process. The HPLC fingerprint evaluation method is important for the quality control of the RSF for the standardization and the TCM that contains the RSF.

## RESULTS AND DISCUSSION

### Optimization of the Extraction Procedure

Sample pretreatment is important for the HPLC analysis of the alkaloids. To eliminate the background contamination and attain the selective extraction of quinolizidine alkaloids, based on the character of the alkaloids, first hydrochloric acid was used to give the alkaloid salts and then sodium hydroxide was used to give the free alkaloids; chloroform was used as extraction solvent because the quinolizidine alkaloids dissolved well in chloroform. The total alkaloids are extracted exclusively and completely. Reproducible HPLC fingerprints can be obtained for the quinolizidine alkaloids under such well-controlled extraction conditions.



#### *Sample Extraction Rate of the Alkaloids*

After purifying, the number of chromatographic peaks was reduced and the peaks due to impurities disappeared. In addition, more than 95% of the main alkaloids were extracted using chloroform (ammonia water) as the extraction solvent. The sample was extracted completely basically. The experimental results are listed in the Table I.

#### *Optimization of Sample Extraction Conditions for the Alkaloids*

The alkaloids were extracted completely after 30 min upon treatment with ultrasound. The sample extraction methods employed used ultrasound and reflux (with chloroform (ammonia solution) and methanol as extraction solvents in both cases). The extraction times were in the range 15–60 min (Table II; for all of the other pretreatments see the above description). Based upon the preliminary results, ultrasound (30 min) with chloroform as solvent was used to obtain quantitative comparisons of the HPLC fingerprints generated by the assay method.

### Qualitative and Quantitative Determinations

The RSD of this analytical method on the basis of the peak-areas of **1**–**9** for six replicate injections was 0.6–1.5%, and the variation of the retention time of each peak was less than 1.5% ($n = 6$). The RSD of the stability test and the precision test was 0.9–1.5%. The fingerprint analysis was especially useful in performing the stability test. The alkaloids are stable and the peak-area of the compounds does not change significantly.

TABLE I Extraction of the alkaloids ($n = 3$)

| *Method* | *Peak 2* | *Peak 3* | *Peak 5* |
|---|---|---|---|
| Before purifying | 100 | 100 | 100 |
| After purifying (relative peak area) | 95.2 | 96.2 | 94.6 |

TABLE II Effect of extraction method, extraction solvent and extraction time ($n = 3$)

| | *Extraction* | | | | |
|---|---|---|---|---|---|
| *Method* | *Solvent* | *Time (min)* | *Peak 2* | *Peak 3* | *Peak 5* |
| Reflux | MeOH | 30 | 100 | 100 | 100 |
| | MeOH | 60 | 98.6 | 97.3 | 97.6 |
| | $CHCl_3$ | 30 | 111.5 | 110.8 | 110.2 |
| | $CHCl_3$ | 60 | 103.1 | 102.4 | 102.8 |
| Ultrasonic | MeOH | 15 | 105.4 | 107.1 | 106.6 |
| | MeOH | 30 | 107.3 | 108.2 | 106.4 |
| | $CHCl_3$ | 15 | 108.1 | 109.0 | 108.5 |
| | $CHCl_3$ | 30 | 114.2 | 115.4 | 113.9 |

The respective retention times ($t_R$/min) of alkaloids chromatogram (Fig. 2) are: for **1**: 3.9 ± 0.1; **2**: 25.0 ± 0.1; **3**: 5.9 ± 0.1; **4**: 9.1 ± 0.1; **5**: 12.9 ± 0.1; **6**: 18.9 ± 0.1; **7**: 21.2 ± 0.1; **8**: 24.7 ± 0.2; **9**: 26.2 ± 0.2.

Calibration curves for nine alkaloids were prepared for the present study. The coefficient of correlation of the reference standard alkaloids was 0.999. The experimental results are listed in Table III.



## Identification of Chromatographic Peaks

To increase the reliability of the most representative quinolizidine alkaloids, nine chromatographic peaks were identified by referring to standard compounds, HPLC/MS analysis and comparison of the physicochemical data of the isolated peak with the literature.

### *Standard Compounds Chromatograms of the Alkaloids*

A mixture of standard compounds of nine alkaloids was analyzed according to the same HPLC conditions as for the samples. Based on comparisons with the retention times of respective peaks between standards and samples, the chromatographic peaks were identified as compounds **1**–**9**: sophoranol, oxymatrine, oxysophocarpine, *N*-methylcytisine, anagyrine,

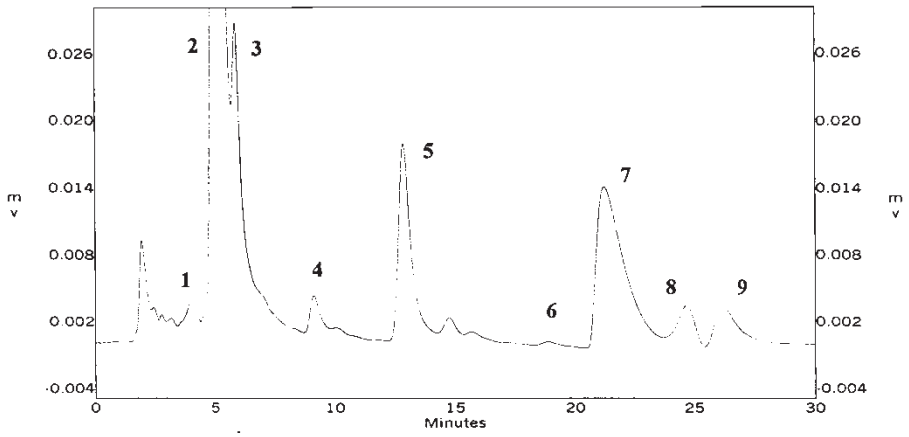


FIGURE 2 HPLC chromatogram of RSF alkaloids extracts. (Peak labels correspond to compound numbering in Fig. 1, for chromatographic conditions, see HPLC conditions.)

TABLE III Alkaloids contents (mg g$^{-1}$) of RSF from different Chinese sources ($n = 3$)

| *No.* | *Location* | *Route* | *1* | *2* | *3* | *4* | *5* | *6* | *7* | *8* | *9* | *Total* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $C_1$ | Liaoning | Market | 0.65 | 28.21 | 15.11 | 2.02 | 4.48 | – | 2.70 | 0.94 | 1.05 | 55.16 |
| $C_2$ | Sichuan | Market | 0.61 | 30.84 | 13.17 | 1.94 | 2.98 | – | 2.58 | 1.04 | 2.31 | 55.47 |
| $C_3$ | Jilin | Market | 0.84 | 32.46 | 21.13 | 2.96 | 1.71 | 0.52 | – | 0.59 | 1.48 | 61.69 |
| $C_4$ | Heilongjiang | Market | 0.85 | 33.25 | 12.85 | 1.49 | 3.87 | – | – | 0.77 | 4.13 | 57.21 |
| $C_5$ | Hunan | Market | 1.34 | 16.03 | 10.05 | 3.03 | 3.67 | 0.11 | 0.23 | 1.53 | 5.76 | 41.75 |
| $C_6$ | Guangdong | Market | 1.25 | 30.11 | 18.42 | 4.31 | 2.04 | 0.21 | 2.42 | 1.24 | 3.67 | 63.67 |
| $C_7$ | Zhejiang | Market | 0.94 | 16.22 | 6.95 | 2.37 | 2.16 | 0.23 | 1.30 | 0.69 | 1.74 | 32.60 |
| $C_8$ | Shanghai | Market | 1.11 | 15.60 | 9.01 | 1.64 | 1.67 | 0.24 | 0.90 | 0.40 | 1.10 | 31.67 |
| $C_9$ | Hebei | Market | 0.91 | 33.26 | 17.59 | 3.11 | 2.48 | 1.51 | – | 2.41 | 0.58 | 61.85 |
| $C_{10}$ | Xi'an | Market | 0.67 | 26.32 | 21.12 | 2.58 | 1.46 | – | 1.33 | 1.61 | 1.02 | 56.11 |
| $C_{11}$ | Henan | Market | 1.07 | 18.76 | 14.33 | 1.61 | 1.13 | – | 2.21 | 1.92 | – | 41.03 |
| $C_{12}$ | Guangxi | Market | 0.61 | 31.25 | 19.37 | 1.95 | 2.85 | 1.18 | – | 1.88 | 1.95 | 61.04 |
| $C_{13}$ | Shanxi | Market | 0.81 | 17.31 | 12.41 | 1.94 | 1.84 | 1.71 | – | 1.74 | 1.84 | 39.60 |
| $C_{14}$ | Hubei | Market | 0.74 | 14.38 | 9.14 | 1.11 | 1.21 | 2.17 | 2.33 | 1.62 | 1.58 | 34.28 |
| $C_{15}$ | Neimenggu | Market | 0.97 | 16.38 | 10.26 | 4.12 | 2.45 | 3.12 | 0.78 | 0.97 | 2.89 | 41.94 |

–: undetected.

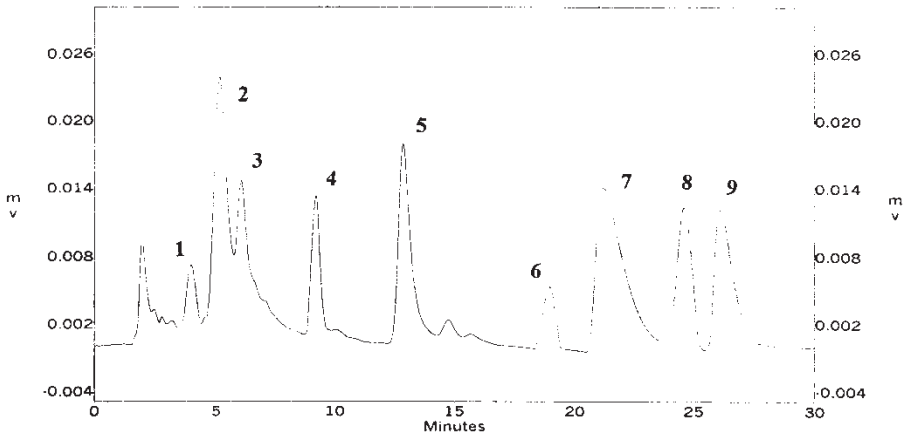


FIGURE 3 HPLC chromatogram of RSF standard compounds of the alkaloids.



sophocarpine, isomatrine, matrine and sophoridine respectively. The chromatogram of the standard compounds of nine alkaloids is shown in Fig. 3.

### *HPLC/MS Analysis to Identify Alkaloids*

To corroborate the structure of the alkaloids, HPLC/MS was utilized to find molecular weight information of correlation peaks of the standard alkaloids. Analysis of the HPLC/MS chromatograms (Fig. 4) shows that the peaks 1–9 are sophoranol ($M_r = 264$), oxymatrine ($M_r = 264$), oxysophocarpine ($M_r = 262$), *N*-methylcytisine, anagyrine ($M_r = 244$), sophocarpine ($M_r = 246$), isomatrine ($M_r = 248$), matrine and sophoridine, respectively, based on the standard compounds and the molecular weight information of the major alkaloids. The peaks 10 ($M_r = 244$), 11 ($M_r = 244$), may be sophoramine, rhombifoline, or *vice versa*, and peak 12 ($M_r = 260$) may be baptifoline based on the molecular weight information of the alkaloids isolated from RSF in the literature [3–5,9–11]. The chromatogram of the HPLC/MS is shown in Fig. 4.

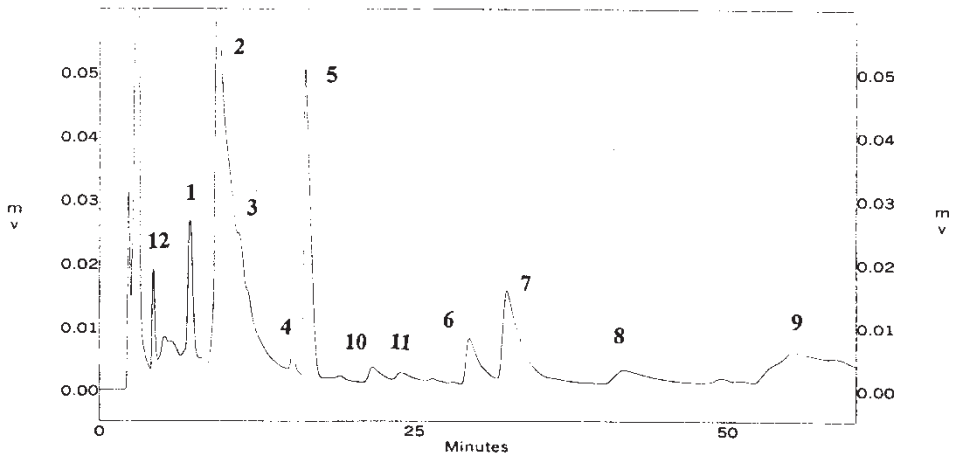


FIGURE 4 HPLC/MS chromatogram of major alkaloids of RSF chromatographic conditions: The flow-rate was kept constant at 0.5 ml/min$^{-1}$ and the solvent system employed was methanol/water/ammonia water 50:50:0.5 (v/v/v); other conditions are the same as before.

### *Identification of the Isolated Peak by Physicochemical Data*

Based on Fig. 2, we collected each chromatographic peak fraction and identified nine peaks. The physicochemical data of the isolated chromatographic peak are:

Peak 1. Colorless needle crystals, mp 152–153°C, $^1$H NMR ($CDCl_3$, *d* ppm): 4.34 (1H, d, 17e-H), 3.80 (1H, m, 11-H), 3.22 (1H, d, 17α-H), 2.88 (1H, d, 10e-H), 2.75 (1H, 2d, 2e-H).

Peak 2. White square crystals, mp 208–209°C. IR (KBr) $\nu_{max}$ ($cm^{-1}$): 2930, 2870(CH), 2280, 1603(lactam), 949(N–O), $^1$H NMR ($CDCl_3$, $\delta$ ppm): 5.09 (1H, q, 11-ß-H), 4.40 (1H, dd, 17-α-H), 2.81–3.17 (3H, 2, 10, 17ß-H).

Peak 3. Colorless edge crystals, mp 207–209°C, $^1$H NMR ($CDCl_3$, $\delta$ ppm): 6.25 (1H, m, 13-H), 5.69 (1H, d, 14-H), 4.85 (1H, m, 11-H), 3.8–4.0 (2H, m, 17-H).

Peak 4. Colorless needle crystals, mp 130–132°C, $^1$H NMR ($CDCl_3$, $\delta$ ppm): 7.27 (1H, dd, 10-H), 6.44 (1H, d, 9-H), 5.99 (1H, d, 8-H), 4.09 (1H, d, 13α-H), 3.89 (1H, d, 13β-H), MS: 204($M^+$), 146, 117, 96, 58(base peak), 42.

Peak 5. Colorless needle crystals, mp 180–181°C, $^1$H NMR ($CDCl_3$, $\delta$ ppm): 7.11 (1H, dd, 10-H), 6.20 (1H, d, 9-H), 5.75 (1H, d, 8-H), 3.92 (1H, d, 13α-H), 3.69 (1H, d, 13β-H).

Peak 6. Colorless needle crystals, mp 51–53°C, $^1$H NMR ($CDCl_3$, $\delta$ ppm): 6.48 (1H, m, 13-H), 5.88 (1H, d, 14-H, $J = 8$ Hz), 4.16 (1H, dd, 17α-H), 3.18 (1H, t, 17β-H), 4.01 (1H, 11-H), 2.85 (2H, 2, 10β-H).

Peak 7. White edge crystals, mp 132–134°C, $^1$H NMR ($CDCl_3$, $\delta$ ppm): 3.18 (1H, 11-H), 3.56–3.76 (2H, 17-H), MS: *m/z*248 ($M^+$), 247 (M–1, base peak), 205, 177, 150, 136, 96.

Peak 8. White edge crystals, mp 73–75°C, $^1$H NMR ($CDCl_3$, $\delta$ ppm): 4.4 (1H, dd, 17α-H), 3.8 (1H, q, 11β-H), 2.64–3.24 (3H, 2,10, 17β-H).

Peak 9. White needle crystals, mp 104–105°C, $^1$H NMR ($CDCl_3$, $\delta$ ppm): 3.22–3.49 (3H, m, 11,17-H), 2.75–2.95 (2H, m, 10α, 2-H), 2.12–2.5 (3H, m, 10β, 2, 14-H).

Based on the physicochemical data and comparisons with the literature [3–5,9–11], the chromatographic peaks 1–9 was identified as sophoranol, oxymatrine, oxysophocarpine, *N*-methylcytisine, anagyrine, sophocarpine, isomatrine, matrine and sophoridine, respectively.

## Fingerprint Evaluation of Various Commercial Radix Sophorae Flavescentis

The RSF has been widely used in traditional medicine in China for centuries. Currently a great deal of RSF is used each year in the China alone. Due to its medicinal and commercial importance it is imperative to set up a rapid and accurate analytical method to evaluate the quality of the commercial RSF. Traditionally the quality of a herbal medicine can be judged by carefully inspecting the morphological and histological characteristics and the surface features. However, we found that there are no recognized differences between the commercial RSF collected from different sources. In the past, the TLC method has been used to evaluate the quality of RSF [12]. This method, described in the Chinese Pharmacopoeia, is not very specific and permits only an approximate estimation of the total alkaloids in plants. A detailed determination of the qualitative and quantitative composition of the alkaloids is not possible. In the present study, we have set up a qualitative and quantitative method by using HPLC.

The chromatograms of the HPLC separation can be used with chromatographic parameters for fingerprint evaluation of commercial RSF. The chromatographic parameters in particular can be used not only for quantitative analysis but also for qualitative distinction from other herbs containing quinolizidine alkaloids-even those from the Radix Sophorae Tonkinensis.

TABLE IV Quantitative analysis data of the standard alkaloids

| *Standard* | *Calibration curve* | *Y* | *Dynamic range (5 point) (μg)* |
|---|---|---|---|
| **1** | $Y = 13341x + 1303$ | 0.999 | 1.0–5.0 |
| **2** | $Y = 14624x + 1242$ | 0.999 | 2.0–10.0 |
| **3** | $Y = 15937x + 1084$ | 0.999 | 2.0–10.0 |
| **4** | $Y = 12985x - 655$ | 0.999 | 1.0–5.0 |
| **5** | $Y = 13463x + 495$ | 0.999 | 1.0–5.0 |
| **6** | $Y = 15382x + 1108$ | 0.999 | 1.0–5.0 |
| **7** | $Y = 11032x - 742$ | 0.999 | 1.0–5.0 |
| **8** | $Y = 11482x - 976$ | 0.999 | 1.0–5.0 |
| **9** | $Y = 12077x + 1033$ | 0.999 | 1.0–5.0 |

*Y*: peak area count; $x$ : injection amount (μg).



Peaks 1, 2, 3, 4, 5 and 8 were relatively rich in RSF and well separated from other peaks, as displayed in their HPLC profile. Therefore, they were chosen for use as the reference to evaluate the commercial RSF. As shown in the HPLC profile, oxymatrine and oxysophocarpine were relatively abundant in the sample from China. Thus, it seems appropriate to use oxymatrine and oxysophocarpine as the index of the quality and quantity of the commercial RSF. After careful comparison of a total of fifteen samples collected from China, we found that samples each had different patterns, as discussed above. Table IV shows the contents of nine alkaloids as determined by HPLC analysis.

After careful comparison of a total of fifteen samples collected from China, we found that the samples each had different patterns. The alkaloid contents of the samples from different sources are quite different. For example, sample $C_4$ did not contain **6** and **7**, sample $C_{11}$ did not contain **6** and **9**. Sample $C_1$, $C_2$, $C_{10}$ did not contain **6**, but the **5** of sample $C_1$ is higher than that of others. In general, based on chromatogram parameters, we can effectively evaluate the quality of the RSF from different sources. The contents of the total alkaloids in samples $C_{1-4}$, $C_6$, $C_9$, $C_{10}$, $C_{12}$ are higher than others. Their quality is also better than others. This can be proved by the results of grading by Hierarchical Cluster (see Table V and Fig. 5) using the software spss 10.0 for windows. The method gives average linkage between groups and the measure type is cosine (standardization has not been carried out for the data).

The similarity is calculated by using the contents of nine alkaloids, and according to a sample similarity of 1.0 from $C_1$ to $C_{16}$ respectively ($C_{16}$ is the average contents of the all samples). Table VI is a similarity matrix obtained by using Euclidean distance as the similarity measure. The results show that the samples are, basically, in accordance no matter which sample similarity is used as 1.0 because all of the similarities are between 0.9 and 1.0.

The relatively low total alkaloid content of the marketed products may result from the processing, which usually involves washing, steaming and drying. We believe such processing may lead to the partial loss of alkaloids. However, we cannot exclude the effect from differences in the environment (climate, soil, *etc*).

TABLE V Grading by hierarchical cluster

| *Grading* | *Sample no.* |
|---|---|
| I | $C_1$, $C_2$, $C_4$, $C_3$, $C_{12}$, $C_9$, $C_6$, $C_{10}$ |
| II | $C_7$, $C_8$, $C_{14}$, $C_{13}$, $C_{15}$, $C_5$, $C_{11}$ |

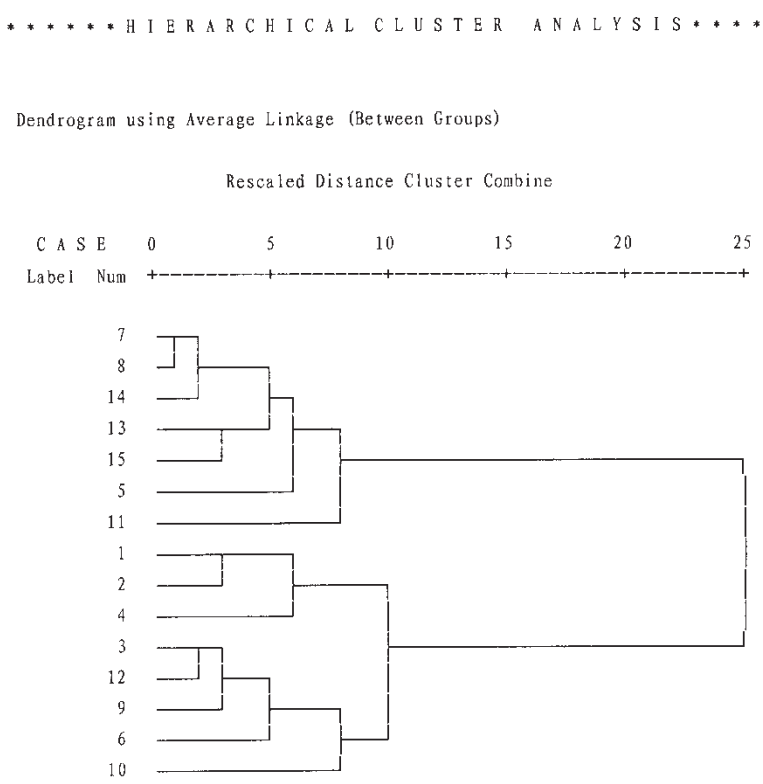


FIGURE 5 Results of hierarchical clustering analysis by using the contents of nine alkaloids.



## HPLC Fingerprint for the Standardization of Phytomedicines

In the standardization of phytomedicines, a direct quantification of the naturally occurring active principles would be desirable. Often this is not possible because most reference compounds are not commercially available and, in addition, in the case of RSF the alkaloids profile is very complex so that analysis for pharmaceutical quality control is rather tedious. However, the obtained alkaloids contents can be correlated with the quality control. A standardization based on these compounds is acceptable from a pharmaceutical viewpoint because they are characteristic constituents in extracts of the plant. We can use this HPLC fingerprint analysis method for the quality control of the RSF and the standardization of phytomedicines.

## EXPERIMENTAL

### Herb Materials

Commercial Radix Sophorae Flavescentis were collected from various markets in China (Liaoning, Sichuan, Jilin, Heilongjiang, Hunan, Guangdong, Zhejiang, Hebei, Henan, Shanxi, Hubei Province and Shanghai, Xi'an City and Guangxi, Neimenggu Municipality). These samples were identified by their external appearance and vouchers of the above samples have been deposited at the Department of Pharmacognosy of Shenyang Pharmaceutical University.

TABLE VI Grading by similarity analysis

| *Proximity matrix* | *Cosine of vectors of values* | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *1* | *2* | *3* | *4* | *5* | *6* | *7* | *8* | *9* | *10* | *11* | *12* | *13* | *14* | *15* | *16* |
| 1 | | 0.994 | 0.998 | 0.985 | 0.956 | 0.991 | 0.991 | 0.996 | 0.992 | 0.978 | 0.981 | 0.992 | 0.981 | 0.982 | 0.968 | 0.995 |
| 2 | 0.994 | | 0.981 | 0.995 | 0.951 | 0.986 | 0.996 | 0.991 | 0.990 | 0.961 | 0.965 | 0.985 | 0.969 | 0.975 | 0.961 | 0.991 |
| 3 | 0.988 | 0.981 | | 0.973 | 0.953 | 0.994 | 0.976 | 0.995 | 0.994 | 0.994 | 0.990 | 0.998 | 0.993 | 0.980 | 0.971 | 0.996 |
| 4 | 0.985 | 0.995 | 0.973 | | 0.957 | 0.977 | 0.992 | 0.984 | 0.985 | 0.947 | 0.946 | 0.980 | 0.962 | 0.961 | 0.956 | 0.984 |
| 5 | 0.956 | 0.951 | 0.953 | 0.957 | | 0.971 | 0.966 | 0.965 | 0.950 | 0.946 | 0.939 | 0.960 | 0.968 | 0.956 | 0.975 | 0.968 |
| 6 | 0.991 | 0.986 | 0.994 | 0.977 | 0.971 | | 0.988 | 0.997 | 0.990 | 0.988 | 0.987 | 0.993 | 0.990 | 0.987 | 0.982 | 0.998 |
| 7 | 0.991 | 0.996 | 0.976 | 0.992 | 0.966 | 0.988 | | 0.991 | 0.987 | 0.957 | 0.960 | 0.981 | 0.970 | 0.975 | 0.975 | 0.990 |
| 8 | 0.996 | 0.991 | 0.995 | 0.984 | 0.965 | 0.997 | 0.991 | | 0.995 | 0.986 | 0.986 | 0.996 | 0.990 | 0.986 | 0.978 | 0.999 |
| 9 | 0.992 | 0.990 | 0.994 | 0.985 | 0.950 | 0.990 | 0.987 | 0.995 | | 0.980 | 0.981 | 0.996 | 0.987 | 0.981 | 0.972 | 0.996 |
| 10 | 0.978 | 0.961 | 0.994 | 0.947 | 0.946 | 0.988 | 0.957 | 0.986 | 0.980 | | 0.997 | 0.990 | 0.991 | 0.978 | 0.963 | 0.987 |
| 11 | 0.981 | 0.965 | 0.990 | 0.946 | 0.939 | 0.987 | 0.960 | 0.986 | 0.981 | 0.997 | | 0.988 | 0.988 | 0.983 | 0.960 | 0.987 |
| 12 | 0.992 | 0.985 | 0.998 | 0.980 | 0.960 | 0.993 | 0.981 | 0.996 | 0.996 | 0.990 | 0.988 | | 0.995 | 0.985 | 0.974 | 0.998 |
| 13 | 0.981 | 0.969 | 0.993 | 0.962 | 0.968 | 0.990 | 0.970 | 0.990 | 0.987 | 0.991 | 0.988 | 0.995 | | 0.988 | 0.985 | 0.993 |
| 14 | 0.982 | 0.975 | 0.980 | 0.961 | 0.956 | 0.987 | 0.975 | 0.986 | 0.981 | 0.978 | 0.983 | 0.985 | 0.988 | | 0.982 | 0.988 |
| 15 | 0.968 | 0.961 | 0.971 | 0.956 | 0.975 | 0.982 | 0.975 | 0.978 | 0.972 | 0.963 | 0.960 | 0.974 | 0.985 | 0.982 | | 0.981 |
| 16 | 995 | 991 | 996 | 984 | 968 | 998 | 990 | 999 | 996 | 987 | 987 | 998 | 993 | 988 | 981 | |

This is a similarity matrix.

## Reference Substances

Nine known quinolizidine alkaloids were identified as sophoranol, oxymatrine, oxysophocarpine, *N*-methylcytisine, anagyrine, sophocarpine, isomatrine, matrine and sophoridine. They all had HPLC purities of 95% or higher and were suitable for assigning HPLC retention times. Physicochemical data can be found in the literature (mp. IR and NMR) [3–5,9–11].

## Chemicals and Solvents

Chloroform, ammonia solution, hydrochloric acid, sodium hydroxide, sodium chloride, anhydrous sodium sulfate, triethylamine (AR grade), methanol (HPLC grade), and distilled water were used.

## Sample Pretreatment

### *Sample Extract*

A crushed sample (1.0000 g) of RSF was extracted by ultrasound with chloroform (50 ml) at ambient temperature (25°C) for 30 min after the medicinal material was drenched with ammonia solution (1.0 ml) for 3 min. The extract was filtered through No.2 Whatman filter paper and the filtrate (20 ml) was evaporated under reduced pressure on a rotary evaporator for 15 ± 3 min during which time the water bath was maintained at 35 ± 2°C.

### *Sample Purifying*

The residue was dissolved with 0.1 mol $l^{-1}$ hydrochloric acid (2 ml) and the mixture filtered through a No. 2 Whatman filter paper and washed 3 times with water (1 ml) each time. Sodium chloride (1 g) and 0.2 mol $l^{-1}$ sodium hydroxide saturated with sodium chloride (2 ml) were added to the filtrate. Then the solution was extracted 5 times with 8, 6, 2, 2, 2 ml chloroform respectively for 5 min each time. The chloroform phase was desiccated with anhydrous sodium sulfate and evaporated on a rotary evaporated under reduced pressure according to the conditions noted above.

### *Sample HPLC Analysis*

The residue was dissolved in the mobile phase and the resultant solution filtered through a 0.45 μm filter. The filtrate was diluted to 10 ml with mobile phase and injected (20 μl) for HPLC analysis.

## Apparatus and Assay Conditions

The HPLC system consisted of a SPD-10Avp delivery system (Shimadzu, Japan), a SPD-10Avp UV-VIS detector (Shimadzu, Japan) and Ana star Baseline data collection software. ESI-MS was by a LCQ model LC-MS instrument (Finnigan, USA). The IR spectra were recorded on a Bruker IFS-55 spectrometer. $^1$H NMR spectra were run on a Bruker AC-250 spectrometer. Reversed-phase conditions utilized a Diamonsil ODS (25 cm × 4.6 mm, I.D: 99903, SN: 803181) column (DIKMA, China), having 5 μm packing.

Separations were achieved with the reversed-phase $C_{18}$ column described above and the solvent system employed was methanol/water/triethylamine 40:60:0.05, (v/v/v). Each sample run was followed by a 15 min re-equilibration.The flow-rate was kept constant at

1.0 ml $min^{-1}$ with the column at 40°C and the peak was monitored at 220 nm; the injection volume was 20 μl. All peaks within the chromatogram could be kept on scale. The area of individual peaks could all be observed and read directly from their respective chromatograms. Nine clearly discernible peaks of interest could be readily determined.

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