# Analysis of *Psoralea corylifolia* L. Fruits in Different Regions

Lu-hua ZHAO, Meng-hua WU, and Bing-ren XIANG*

*Analytic Center, China Pharmaceutical University; Nanjing, 210009, China.*
Received March 26, 2005; accepted May 18, 2005

**Application of multivariate data analysis has become a popular method in the last decades, mainly because it can provide information not otherwise accessible. The information includes classification, searching similarities, finding relationships, finding physical significance to principal components, *etc.* Twenty-two Chinese medicinal herbs containing twelve constituents were collected and determined by HPLC. The results were studied by hierarchical cluster analysis (HCA) and principal components analysis (PCA). It was shown that the samples could be clustered reasonably into three groups, hence corresponding with the typical habitats of *Psoralea corylifolia* L.**

**Key words** *Psoralea corylifolia* L.; hierarchical cluster analysis; principal components analysis; typical habitat

*Psoralea corylifolia*, one of the widely used traditional Chinese medicines (TCM), derived itself from mature and dry fruits of Leguminous plant *Psoralea corylifolia* L. officially listed in the Pharmacopoeia of the Peoples' Republic of China (Edit 2000 Vol II). It has been used in the treatment of enuresis pollakiuria, waist and knee psychroalgia and kidney weak. A variety of biological activities of its constituents or extract have been reported. *P. corylifolia* contained coumarins, flavonoids and merotepenes *etc.* such as psoralen, isopsoralen, neobavaislfoavone, bovachin, bavaislfoavone, bavachromene, psoralidin, corylifolinin, bavachinin, bavachalcone. A lot of experiments were studied on the effective components of *P. corylifolia* about their bioactivity. The clinical applications of psoralen and isopsoralen have been reported for the treatment of skin diseases such as psoriasis and vithigo. They can also make the virus in blood inactive and are commonly used for the treatment of renal weakness and other kidney dis-functions.[1—3] Bavachinin and bavachin show antioxidative activities.[4] Psoralidin shows stronger activities against Gram(−) *Shigeua sonnei* and *Shigeua flexneri*.[5] The fruit extract of *P. corylifolia* is suggested to be useful as the remedy for bone fracture, osteomalacia and osteoporosis also show potent inhibition of mitochondrial and microsomal lipid peroxidation.[4,6]

*P. corylifolia* distributed extensively in China and some other Asian countries such as Vietnam and Burma. It was reported that they had the same components but had some differences in the content of each component. Therefore, an high-performance liquid chromatography (HPLC) method was developed to qualitatively and quantitatively analyze twelve constituents: psoralen, isopsoralen, neobavaislfoavone, bovachin, bavaislfoavone, bavachromene, psoralidin, corylifolinin, bavachinin, unknown compound **1**, bavachalcone and unknown compound **2** in the 70% ethanol extracts of the fruits of *Psoralea corylifolia* collected from different geographical origins. Some regularity was presented. Then the relatively simple and effective multivariate data analysis approach based on hierarchical cluster analysis (HCA) and principal components analysis (PCA) were applied for differentiating *P. corylifolia* in different regions. With the results of these two methods compared the same conclusion was given: *P. corylifolia* in Sichuan province and Henan province used as genuine medicinal drug is reasonable.

## Experimental

**Reagents and Materials** The sample of *P. corylifolia* fruits was collected in China and identified by Professor Song Xuehua of China Pharmaceutical University. The voucher specimens are currently preserved in China Pharmaceutical University.

Acetonitrile (TEDIA U.S.A.) and water were of HPLC grade. Acetic acid was of analytical grade.

**Standards** Standards of psoralen and isopsoralen were provided by the National Institute for the Control of Pharmaceutical and Biological Products, Beijing, China. Other standards, *i.e.* neobavaislfoavone, bovachin, bavaislfoavone, bavachromene, psoralidin, corylifolinin, bavachinin and bavachalcone were extracted from *P corylifolia* by us and confirmed by UV, MS and $^1$H-NMR. Their purity is higher than 98.0%.

**Apparatus and Column** The HPLC system consisted of binary pumps (Shimadzu LC-10AD, Japan), a UV detector (Shimadzu LC-10A vp, Japan), and a model 7725 I manual injector valve with a 20 $\mu$l sample loop. The signals from the detector were analyzed with a computer equipped with a software of N2000 system (Zhejiang University, China). An Alltech $C_{18}$, 250×4.6 mm, 5 $\mu$m, column (Alltima, China) was used for all chromatographic separations. The temperature of the column was kept at room temperature.

**Chromatographic Conditions** The mobile phase was acetonitrile (solvent A) and 0.1% acetic acid–water (solvent B) in the gradient mode: 0—20 min, 60—50% B; 20—35 min, 50—40% B; 35—45 min, 40—30% B; 45—55 min, 30—20% B; 60 min, stop. The flow-rate was 1.0 ml·min$^{-1}$. The effluent was monitored at 245 nm.

**Sample Preparation** The fruit of *P. corylifolia* was ground into fine powder. Then 0.2 g of the powder was weighed accurately and immersed with 20 ml 70% ethanol for half an hour in a conical flask. After ultrasonication for half an hour, it was cooled to the room temperature. Two milliliters of the extract solution was diluted to 10 ml with 70% ethanol, filtered with 0.45 $\mu$m filter as sample solution.

## Results and Discussion

**HPLC Analysis** Twenty microliters of the sample solution were injected into HPLC column and analyzed under above chromatographic condition. All twelve constituents were successfully analyzed in a single run. A preliminary experiment was first conducted with isocratic elution using 60% acetonitrile or 65% acetonitrile at a flow-rate of 1.0 ml/min. In both instances, the twelve compounds gave only eleven peaks, compounds **1** and **2** being overlapped. At 60% acetonitrile, the analysis could be accomplished within 150 min. With 65% acetonitrile, the elution time was 120 min. At the same time, from peak 5 to peak 12, there was a long distance between every two peaks. For the sake of improving the shape of each peak, the water was changed to 0.1% acetic acid. After a series of experiments, it was found

* To whom correspondence should be addressed. e-mail: zhaoLuhua@hotmail.com and xangbr@cpu.edu.cn 

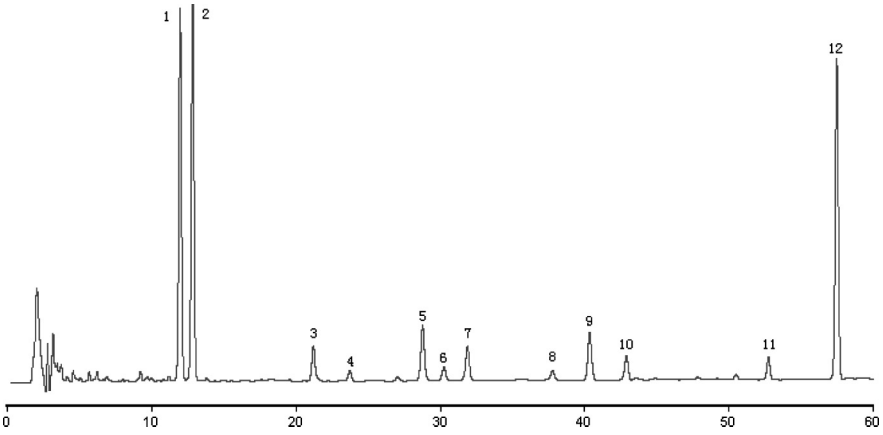


Fig. 1. Chromatograms of the Extract of *Psoralea corylifolia* Fruit

Column: Alltech $C_{18}$, 250×4.6 mm, 5 $\mu$m. Eluents: solvent A: acetonitrile and solvent B: 0.1% acetic acid–water. Elution profile: 0—20 min, 60—50% B (40—50% A); 20—35 min, 50—40% B (50—60% A); 35—45 min, 40—30% B (60—70% A); 45—55 min, 30—20% B (70—80% A); 60 min, stop. Detection: UV at 245 nm. Peak mumbers as follows: 1. psoralen, 2. isopsoralen, 3. neobavaislfoavone, 4. bovachin, 5. bavaislfoavone, 6. bavachromene, 7. psoralidin, 8. corylifolinin, 9. bavachinin, 10. unknown compound **1**, 11. bavachalcone, 12. unknown compound.

Table 1. The Effective Components Relative Content in Psoralea Corylifolia Fruits from Different Habitats

| | Habitat | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Yunnan | 1 | 0.735 | 0.753 | 0.324 | 0.153 | 0.070 | 0.314 | 0.355 | 0.719 | 0.368 | 0.041 | 3.435 |
| 2 | Burma | 1 | 0.758 | 0.731 | 0.328 | 0.191 | 0.075 | 0.386 | 0.363 | 0.738 | 0.389 | 0.041 | 3.542 |
| 3 | Jiangjin, Sichuan | 1 | 1.105 | 0.104 | 0.030 | 0.207 | 0.041 | 0.113 | 0.026 | 0.179 | 0.077 | 0.068 | 0.553 |
| 4 | Fuyang, Anhui | 1 | 0.921 | 0.331 | 0.126 | 0.204 | 0.061 | 0.173 | 0.121 | 0.304 | 0.194 | 0.056 | 1.631 |
| 5 | Hechuan, Sichuan | 1 | 1.050 | 0.116 | 0.055 | 0.213 | 0.060 | 0.120 | 0.038 | 0.148 | 0.076 | 0.075 | 0.360 |
| 6 | Qinyang, Henan | 1 | 0.810 | 0.205 | 0.080 | 0.141 | 0.045 | 0.136 | 0.074 | 0.195 | 0.125 | 0.045 | 1.081 |
| 7 | Vietnam | 1 | 0.807 | 0.506 | 0.241 | 0.140 | 0.055 | 0.242 | 0.255 | 0.521 | 0.276 | 0.035 | 2.556 |
| 8 | Shanxi[a] | 1 | 0.856 | 0.288 | 0.126 | 0.143 | 0.053 | 0.165 | 0.124 | 0.272 | 0.166 | 0.041 | 1.540 |
| 9 | Bo'ai, Henan | 1 | 0.823 | 0.173 | 0.057 | 0.199 | 0.047 | 0.134 | 0.051 | 0.201 | 0.088 | 0.051 | 0.715 |
| 10 | Guangyuan, Sichuan | 1 | 1.081 | 0.130 | 0.051 | 0.243 | 0.065 | 0.128 | 0.028 | 0.180 | 0.104 | 0.096 | 0.382 |
| 11 | Shangqiu, Henan | 1 | 0.782 | 0.205 | 0.081 | 0.087 | 0.026 | 0.124 | 0.101 | 0.250 | 0.115 | 0.022 | 1.115 |
| 12 | Liu'an, Anhui | 1 | 0.834 | 0.356 | 0.112 | 0.165 | 0.054 | 0.287 | 0.170 | 0.343 | 0.209 | 0.063 | 2.039 |
| 13 | Xingping, Shanxi[aa] | 1 | 0.956 | 0.389 | 0.124 | 0.194 | 0.057 | 0.232 | 0.150 | 0.358 | 0.242 | 0.077 | 2.221 |
| 14 | Dujiangyan, Sichuan | 1 | 0.806 | 0.210 | 0.090 | 0.050 | 0.020 | 0.141 | 0.119 | 0.247 | 0.109 | 0.014 | 1.058 |
| 15 | Yunnan | 1 | 0.821 | 0.568 | 0.190 | 0.137 | 0.051 | 0.283 | 0.228 | 0.465 | 0.321 | 0.046 | 3.076 |
| 16 | Liu'an, Anhui | 1 | 0.841 | 0.373 | 0.136 | 0.172 | 0.061 | 0.238 | 0.185 | 0.366 | 0.204 | 0.057 | 2.096 |
| 17 | Jintang, Sichuan | 1 | 0.810 | 0.214 | 0.072 | 0.104 | 0.038 | 0.147 | 0.113 | 0.258 | 0.135 | 0.042 | 1.271 |
| 18 | Xinxiang, Henan | 1 | 1.114 | 0.128 | 0.035 | 0.223 | 0.063 | 0.154 | 0.049 | 0.198 | 0.100 | 0.091 | 0.995 |
| 19 | Xinyang, Henan | 1 | 0.827 | 0.246 | 0.085 | 0.122 | 0.039 | 0.185 | 0.103 | 0.280 | 0.155 | 0.040 | 1.164 |
| 20 | Fuyang, Anhui | 1 | 0.949 | 0.361 | 0.119 | 0.219 | 0.062 | 0.256 | 0.139 | 0.324 | 0.226 | 0.079 | 2.052 |
| 21 | Guangyuan, Sichuan | 1 | 0.938 | 0.173 | 0.054 | 0.105 | 0.028 | 0.118 | 0.064 | 0.214 | 0.099 | 0.030 | 0.836 |
| 22 | Dujiangyan, Sichuan | 1 | 1.071 | 0.123 | 0.030 | 0.213 | 0.055 | 0.130 | 0.037 | 0.183 | 0.086 | 0.076 | 0.960 |

that linear-gradient elution with the profile given in previous Section separated all the compounds within 60 min. The relative content (the ratio of peak area of sample constituents to the peak 1) was studied by PCA and HCA. Figure 1, showing the separation of the twelve constituent with the following retention times: 1, 11.6; 2, 12.5; 3, 0.3; 4, 22.8; 5, 27.8; 6, 29.2; 7, 30.9; 8, 36.7; 9, 39.2; 10, 41.8; 11, 51.9; 12, 56.7. Table 1, showing the results of the experiment.

**Hierarchical Cluster Analysis (HCA)** HCA is an unsupervised technique that examines the inter-point distances between all of the samples and represents that information in the form of two-dimensional row spaces in a form which facilitates the use of human pattern-recognition abilities.

To generate the dendrogram, HCA methods form clusters of samples based on their nearness in row space. A common approach is to initially treat every sample as a cluster and join closest clusters together. This process is repeated until only one cluster remains. Variations of HCA use different approaches to measure distances between clusters.

Eight hierarchical cluster analysis methods such as Ward's average distance and Ward's minimum distance *etc*. were used to analyze the data of the relative content of effective components in *P. corylifolia* fruits from different habitats. Here Ward's average distance hierarchical cluster analysis produced the better classification. The dendrogram was presented in Fig. 2. From the dendrogram, these samples could be grouped into two kinds D and C. Group D was clustered by group A and group B.

As far as their geographical distribution was concerned, the samples in group A were from Henan province and Sichuan province, the samples in group B were from the provinces of Shanxi[aa], Shanxi[a] and Anhui, the samples in group C were from Yunnan province, Vietnam and Burma. As for the climate, Sichuan is a sub-tropic province. Henan

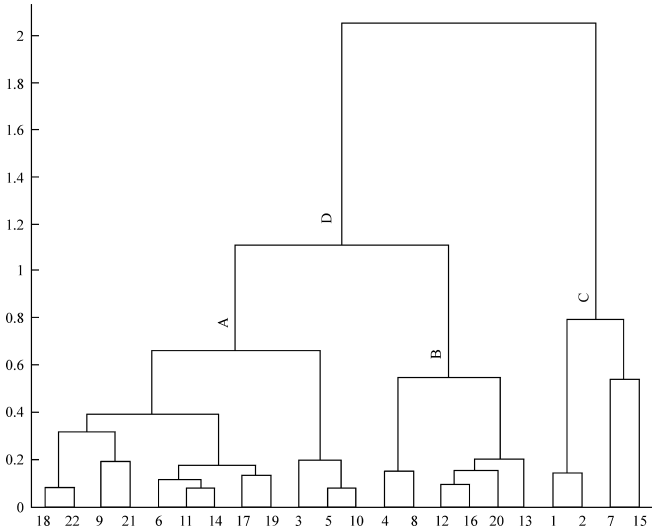


Fig. 2. Dendrogram Using Ward's Average Distance Hierarchical Cluster Analysis

The relative content=the peak area of effective component/the peak area of psoralen (No. 1). 1—12 are the same with the No. in Fig. 1.

1 to 22 are different habitats. The samples in group A were from the province of Henan and Sichuan, the samples in group B were from the province of Shanxi[aa], Shanxi[a] and Anhui, the samples in group C were from Yunnan, Vietnam and Burma.

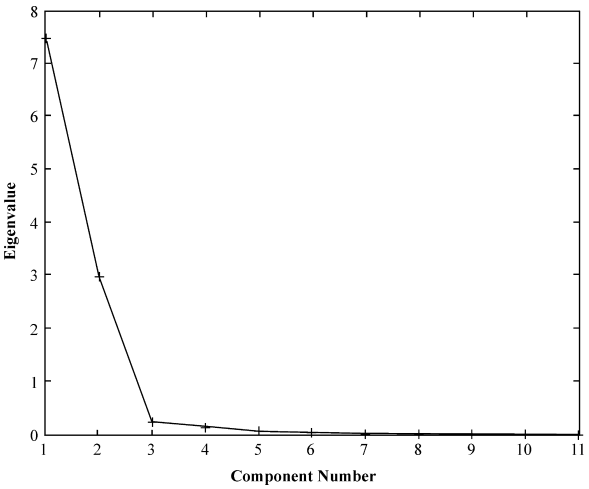


Fig. 3. Every Principal Component Contributes to the Result after Autoscaled

province is located in the boundary between warm-temperate zone and sub-tropic zone. These two provinces belong to group A. Group B has the distribution in warm-temperate zone and temperate zone including provinces of Shanxi[aa], Shanxi[a] and Anhui. Group C are mainly distributed in tropical zone such as Yunnan province, Vietnam and Burma. On the basis of the information above, the growth of natural drugs are affected by temperature, exposure to sunlight and moisture *etc.*[7] *P. corylifolia* is a genuine medicinal drug of Sichuan and Henan provinces over the period of several thousand years.[8] From the dendrogram, we can see the result is well matched with the typical habitats of *P. corylifolia*.

**Principal Components Analysis (PCA)** PCA is a mathematical manipulation of a data matrix where the goal is to represent the variation present in many variables using a small number of "factors". A new row space is constructed in which to plot the samples by redefining the axes using factors rather than the original measurement variables compared with HCA.

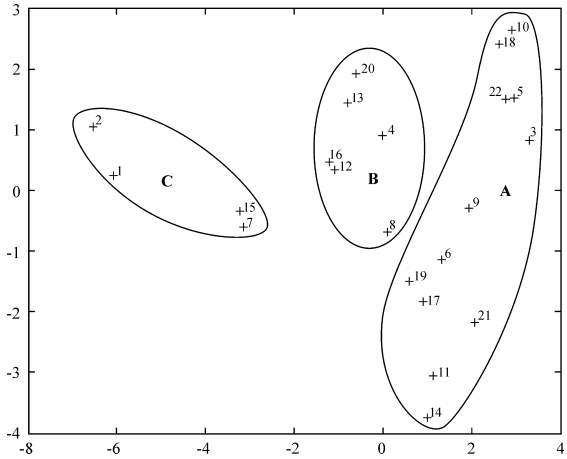


Fig. 4. Scores Plots for the Studied Species When Choosing the First and the Second Principal Component

Data had to be auto-scaled since not all the variables had the same scale. After auto-scaling, the original variables (the relative content) were substituted with new components. These new components were principal components which may represent a comprehensive property of the original variables. Figure 3 presented that every principal component contributed to the result after auto-scaling. The contribution of the first and the second principal component added up to 94% also, so they were selected in PCA. Figure 4 presented the scores plot for the studied species when choosing the first and the second principal component. From the plots these samples could also be grouped into three kinds (symbol A, B, C have the same meaning as in HCA dendrogram).

## Conclusion

Both HCA and PCA can produce the same reasonable results although all variables were used to analyze the data in HCA while only two principal components in PCA. We can conclude that the main classification pattern is caused by their native geographical distribution. All the 22 HPLC data sets were studied using Ward's average distance hierarchical cluster analysis and PCA to reveal the relationship between these samples and between samples and their geographical distribution. It successfully explained that Sichuan province and Henan province were typical habitats of *P. corylifolia* and could be useful to guide genuine medicinal drugs cultivation.

**Acknowledgements** This research was supported by Hi-Tech Research and Development Program of China, Ministry of Science and Technology of the People's Republic of China. (No. 2002 AA2Z3214). We thank Professor Sheng Longsheng for critically reviewing the manuscript.

**References**

1) Jiangsu New Medical College, "Zhong Yao Dacidian (Chinese Herb Dictionary)," ed. by Science and Technology Publishing Co., Shang-Hai, 1993.
2) Chakraborty D. P., Roy S., Chahraborty A. K., *Pigm. Cell Res.*, **9**, 107—116 (1996).
3) Xu J., *Chinese Journal of Disinfection*, **13**, 92—96 (1996).
4) Hiroyuki H., Junji I., Yukiyoshi T., *Phytother. Res.*, **16**, 539—544 (2002).
5) Naznin A., Khatune M., Ekramul Islam M., Ekramul Haque, Khond-

kar P., Mukhlesur Rahman M., *Fitoterapia*, **75**, 228—230 (2004).
6) Miura H., Nishida H., Linuma M., *Planta Med.*, **62**, 150—153 (1996).
7) Huang T. K., Zhao H. B., "Geography of Natural Drugs," ed. by Publishing House of Medical Science of China, Beijing, 1993.
8) Editorial Committee for Chinese Bencao of State Administration of Traditional Chinese Medicine of P. R. C, "Chinese Bencao," ed. by Science and Technology Publishing Co., ShangHai, 1999, pp. 403—409.