Cold-hot Nature Distinguish of Traditional Chinese Medicinal Herbs by Discriminant Analysis of $^1$H-NMR Spectral Data

Hong L$^{1,a,#}$, Qian XU$^{1,b,#}$, Jie ZHANG$^{1,c}$ and Hui XU$^{1,2,3,d,*}$

$^1$School of Pharmacy, Yantai University, Yantai, Shandong, PR China

$^2$Key Laboratory of Molecular Pharmacology and Drug Evaluation, Yantai University, China

$^3$Collaborative Innovation Center of Advanced Drug Delivery System and Biotech Drugs in Universities of Shandong, Ministry of Education, Yantai, China

$^a$7853539502@163.com, $^b$mogumini@126.com, $^c$zhangjie1886551@163.com,$^d$xuhui33@sina.com

$^*$Corresponding author

Keywords: Chinese Medicinal Herbs, Cold-Hot Nature, $^1$H-NMR, Discriminant Analysis.

Abstract. “Cold-hot nature” is the key part of the theory on herb properties in traditional Chinese medicine (TCM). Traditionally, the cold-hot natures of medicinal herbs were ascertained mainly dependent on medicinal experience. Nowadays systematic methods considering the material basis from a variety of chemical substances are needed and may be reliable to differentiate cold-hot nature of various herbs. In the present study, nuclear magnetic resonance spectroscopy of proton ($^1$H-NMR) was combined with pattern recognition techniques for cold-hot nature classification of Chinese medicinal herbs for the first time. $^1$H-NMR determination was carried out on 62 kinds of Chinese medicinal herbs. Canonical discriminant functions (CDF) and fisher discriminant analysis (FDA) then were applied to analyze the large $^1$H-NMR spectra data set obtained. The results indicated that the combination of $^1$H-NMR and multivariate statistical analysis may provide a more efficient means for the research and exploration of cold-hot nature of medicinal herbs.

Introduction

During the long practice history of traditional Chinese medicine (TCM), herbal medicines have been effectively used to eliminate pathogenic factors, restore normal function of the internal organs and then enable the body to recover from illness. The theory on herb properties are pharmacology of Chinese herbs, among which four-nature theory concerned with cold, hot, warm and cool nature of herbs is one of the main contents. The four major properties can be summed up as cold and hot[1]. As highly abstracted TCM theory, the cold-hot property of CMMs is still not fully understood and remains to be elucidated by systems biology approach.

The classification of cold and hot properties of CMMs is derived from the responses of the body to the CMM and also the effects of herbs observed by ancient TCM physician. “To treat cold syndrome with hot, and to treat heat syndrome with cold herbs” has become the basic principle of using Chinese herbs in TCM[2]. So far, four natures of herbs are determined by their curative effects on cold or heat syndromes. However, it suffers a serious credibility problem, especially in the West, for the lack of rigorous randomized trials in support of TCM herb treatments and validation for Four-nature theory. On the other hand, it is difficult to
determine nature for all herbs by this means because of large expenditures of money and time[3]. Thus how to reasonably reveal the relationship between herbs’ cold-hot nature and material basis and then give unambiguous nature distinguish is of great importance for the development and worldwide acceptance of TCM[4].

It may be a viable problem solving mode to model and predict nature of herbs by combining advanced biological and chemical testing with data mining techniques. Metabolomics is a promising systems biology method to profile entire endogenous metabolites and monitor their fluctuations related to an exogenous stimulus. Thus, metabolomics approach was applied to characterize the cold and hot properties of CMMs[5]. The purpose of the present study is to develop some model for distinguishing the cold-hot nature of Chinese herbal medicines using spectroscopic methods coupled with classic pattern recognition techniques. Here we report some results based on 1H nuclear magnetic resonance spectroscopy (1H-NMR) of herbs and the classic pattern recognition techniques, Canonical discriminant functions (CDF) and Fisher discriminant analysis (FDA).

Materials and Methods

Herb Materials and Extraction

The Chinese medicinal herbs with undisputable cold-hot nature involved in the present study included 31 cold ones and 31 hot ones, which were showed in Table 1. The air-dried medicinal section of each herb was collected from indigenous source place in China. All samples were authenticated by Prof. Feng Li and voucher specimens were deposited in the Department of Chinese Herbology, Shandong University of TCM, Jinan, China. Each sample (80 g) was powdered or chopped and then extracted with distilled water under reflux (2 × 1000 mL, once 1 h). The combined water extracts were evaporated in vacuum to yield a brown residue and then freeze-dried and grinded to fine powder in a pestle and mortar, and then stored at -20°C until analysis.

<table>
<thead>
<tr>
<th>Chinese name</th>
<th>Latin name</th>
<th>Chinese name</th>
<th>Latin name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ce-bai-ye</td>
<td>CacumenPlatycladi</td>
<td>Luo-shi-teng</td>
<td>Trachelospermumjasminoides</td>
</tr>
<tr>
<td>Chai-hu</td>
<td>Radix bupleuri</td>
<td>Xi-xian-cao</td>
<td>HerbaSiegesbeckiae</td>
</tr>
<tr>
<td>Che-qian-zi</td>
<td>Plantaginis semen</td>
<td>Tian-dong</td>
<td>Radix Asparagi</td>
</tr>
<tr>
<td>Da-huang</td>
<td>Radix et rhizome rhei</td>
<td>Qin-pi</td>
<td>Cortex fraxini</td>
</tr>
<tr>
<td>Da-qing-ye</td>
<td>Folium isatidis</td>
<td>Pu-gong-ying</td>
<td>Herbaraxaci</td>
</tr>
<tr>
<td>Dan-zhu-ye</td>
<td>Herbalophatheri</td>
<td>Qu-mai</td>
<td>Dianthus superbus Linn.</td>
</tr>
<tr>
<td>Di-fu-zi</td>
<td>Kochiascoparia L. Schrad.</td>
<td>Zhi-mu</td>
<td>Rhizomaanemarrhenae</td>
</tr>
<tr>
<td>Fang-jia</td>
<td>Radix stephanieetetandrae</td>
<td>Zhi-zi</td>
<td>Fructusgardeniae</td>
</tr>
<tr>
<td>Gan-sui</td>
<td>Euphorbia kansui T.N.Liou ex S.B.Ho</td>
<td>Zi-cao</td>
<td>Lithospermumerythrorhizon Sieb.et Zucc.</td>
</tr>
<tr>
<td>Gua-lou</td>
<td>Trichosantheskirilowii Maxim</td>
<td>Chuan-bei-mu</td>
<td>Fritillariacirrhosa D. Don</td>
</tr>
<tr>
<td>Hai-zao</td>
<td>Sargsam</td>
<td>Ge-gen</td>
<td>Radix puerariaeolobatae</td>
</tr>
<tr>
<td>Huang-bo</td>
<td>Cortex phellodendri</td>
<td>Han-lian</td>
<td>HerbaEcliptaeEcliptapostrala L.</td>
</tr>
<tr>
<td>Huang-lian</td>
<td>Rhizomacoptidis</td>
<td>Bo-he</td>
<td>Herbamenthae</td>
</tr>
<tr>
<td>Jin-yin-hua</td>
<td>Floslonicarae japonicae</td>
<td>Nv-zhen-zi</td>
<td>FructusLigustriLucidi</td>
</tr>
<tr>
<td>Long-dan(cao)</td>
<td>Gentianasacabra Bunge</td>
<td>Di-huang</td>
<td>Radix rehmanniae</td>
</tr>
<tr>
<td>Lu-hui</td>
<td>Aloe vera var. chinensis(Haw.) Berg</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
1H-NMR Data Collection

Freeze-dried sample with a weight equivalent to 200 mg of raw herbal medicine was weighed into an Eppendorf tube. Then 1 mL of dimethyl sulfoxide (DMSO) -d6 containing 0.05% tetramethylsilane (TMS) was added to each sample and mixed thoroughly and sonicated at room temperature for 10 min. The sample then was centrifuged at 3,000 rpm for 5 min and the supernatant was used for NMR analysis. All one-dimensional 1H-NMR spectra were acquired on a pulse Fourier transform nuclear magnetic resonance spectrometer (Bruker AV-400, Germany) at 400.13 MHz using an NMR software package of XWIN-NMR3.5 and Z-axis gradients. Samples were measured in 5-mm-o.d. tubes and at room temperature. Typical acquisition parameters included 65536 data points, a spectral width of 13966 Hz, a pulse interval of 1.00 s, and an acquisition time of 2.35 s. For the standard 1D spectrum an exponential line-broadening function of 0.3 Hz was applied to the free induction decay prior to Fourier transformation. All spectra were processed for phase and baseline correction and chemical shifts (δ) were given in ppm with TMS as a reference (δ 0.00).

Data Reduction and Multivariate Statistical Analysis

MestReNova NMR processing software (ver. 5.2.3, Mestrelab Research) was used to integrate spectral regions of equal bucket size of 0.01 ppm width within the chemical shift range of δ-0.03-10.00. The two spectral regions of δ-0.03-0.03 and δ2.40-2.60 were excluded to eliminate residual signals of TMS and DMSO, respectively. Data normalization was performed by scaling spectral intensity of each region to TMS. All the spectral data were rearranged in such a way that the rows of data matrix represented the herbs (subjects) and the columns represented chemical shifts (variables). The cold-hot nature affiliation was used as a dependent variable, i.e., the variable to be predicted by pattern recognition method. CDF and FDA then were performed on the 1H-NMR spectra data set obtained using the SPSS program (ver. 13.0, SPSS, Chicago, IL) and PAST, a free statistics software by Hammer and Harper (ver. 1.30, University of Oslo).
Results and Discussion

The cold-hot property of Chinese material medica (CMM) and the application of its corresponding knowledge in the diagnosis, differentiation and treatment of diseases have been considered to be the extremely important part of traditional Chinese medicine (TCM). Up to now, classifying cold-hot nature of Chinese medicinal herbs is still difficult and mainly dependent on traditional experiences of TCM[1,2]. Owing to the fact that cold-hot nature of herb originates from its multitudinous chemical substances, systematic methods considering a variety of metabolites may be reliable to differentiate cold-hot nature of various herbs. 1H-NMR is one of the principal techniques used to obtain chemical structure information about organic molecules in solution and solid state due to the chemical shift and Zeeman effect on the resonant frequencies of proton. Recently, 1H-NMR has been combined with chemometric methods to metabolomically profile several types of plants and plant functional genomes, as well as in a fingerprinting tool for assessing the quality of natural products[5].

Fisher discriminant analysis (FDA) is a linear dimensional reduction technique widely used for dimension reduction and pattern classification. As a supervised clustering method, the aim of FDA is to find the optimal Fisher discriminant vector such that the Fisher criterion function is maximized. FDA makes full use of fault classification information so as to more effectively separate the normal data set from the fault data sets in the normal process situation[6]. Therefore, FDA was performed by PAST in the present study to enhance the poor separation between the classes under investigation obtained with the CDF model.

In what concerned the data set of 62 Chinese medicinal herbs, a better cold-hot nature class separation was attained after applying FDA for the integrated 1H-NMR spectra. As showed in Fig. 1, 62 Chinese medicinal herbs were analyzed with canonical discriminant functions (CDF) of 1H NMR spectra data set. As a result, the cold-hot nature classes of Chinese medicinal herbs could be discriminated by CDF with the accuracy up to 90% (92.1%), but nv-zhen-zi, chuan-bei-mu and rou-gui were misjudged. The scores plot shown in Fig. 2 indicated that the herbs got plus scores were classified as cold ones, while those with minus scores as hot ones. As a result, the cold-hot nature classes of Chinese medicinal herbs could be discriminated by
FDA with the accuracy up to 90% (95.24%), but dan-zhu-ye, fang-ji, huang-bo, nv-zhen-zi and gao-ben were misjudged. Nv-zhen-zi, the herb experientially belonging to cold nature, was classified as hot by FDA and CDF respectively. This result showed that each statistical analysis method had different kinds of disadvantage, there should be a variety of statistical analysis to reduce the system error and improve accuracy of the results.

As a result, the cold and hot properties of 62 Chinese material medica were identified or tentatively identified under the guidance of ¹H-NMR method coupled with multivariate statistical analysis. This paper partly reveals the cold and hot properties of Chinese material medica with analytical approaches.

![Figure 2. Fisher discriminant analysis (FDA) scores plot of TCM herbs with different nature](image)

H&L represents cold; W&R represents hot.

**Conclusions**

In the present study, the classic pattern recognition methods, CDF and FDA, were applied to analyze the large ¹H-NMR spectra data set obtained for various metabolites of Chinese medicinal herbs for cold-hot nature distinguish for the first time. The results indicated that ¹H-NMR method coupled with multivariate statistical analysis may be a promising tool for distinguishing the cold-hot nature of different Chinese medicinal herbs. Future work would concentrate on further optimizing the nature classification and prediction, assigning the peaks using loading plot analysis. Moreover, the variety of samples studied would be further enlarged in order to ensure the statistical conclusion credibility. In conclusion, this study provides a successful model for rapid and efficient identification cold and hot properties of Chinese material medica. Meanwhile it also provides beneficial information for the similar research in traditional Chinese medicine.

**References**


