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Determination of Metallic Elements for Quality Control of Herbal Medicines

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Herbal medicines contain multiple compounds, each of which may be relevant to the medicine's putative activity. Therefore, fingerprint techniques that look at a suite of compounds, including their respective ratios, provide a more powerful approach to the quality control of herbal medicines. A new method called spectroscopic fingerprint, which is accurate and rapid, was proposed in this paper. Some typical examples of herbal medicines were presented. Fifteen metallic elements in sixteen herbal medicines samples were measured by induced coupled plasma-atomic emission spectroscopy (ICP-AES). The data analysis was performed with the softwares MATLAB and EXCEL. The experimental results clearly demonstrated that the proposed method was a useful tool to control the quality of herbal medicines.

Key Words: Herbal medicine, Quality control, Spectroscopic fingerprint, ICP-AES, Metallic elements.

INTRODUCTION

Herbal medicines including Chinese herbal medicine and other folk medicines are being used by more and more people nowadays in the whole world for improving health condition of human beings as well as preventing and healing diseases. Unlike the single chemical entity that forms the basis of modern pharmacology and drug development, the paradigm of herbal medicine consists of hundreds of ingredients. For most of these ingredients, very little knowledge has been obtained about their chemical compositions and characters. Because the activity of herbal medicines is due to more the overall ingredients than a single constituent, the method to control the quality of chemical synthetic drug is not fit to herbal medicines. More examples of the synergic effects of chemical ingredients in herbal medicines have been reported[1]. Selection of individual analytical compound for determining either efficacy or quality offends against herbal medicine principles[2]. In some eastern countries such as China, Japan and Korea, the identification of herbal medicines mainly depends on the difference of the appearance and odor of the plants, which could only be figured out by the experienced herbalist doctors. This traditional practice has greatly prevented the herbal medicines from spreading around the whole world, because modern medical science is mostly based on chemical theory and advanced instrument. In many western countries, the herbal medicines have still been treated as food by the local governments. This problem urges the herbal medicines researchers to focus on establishing quality control standards for raw materials and the standardization of finished herbal drugs. This difficulty has been acknowledged in the draft of a strategic plan for regional traditional medicine issued by the World Health Organization (WHO)[3]. In recent years, a new method called 'chromatographic fingerprint analysis' was proposed to solve this problem[4]. It uses modern chromatographic techniques, such as GC, HPLC and HPTLC, to construct specific patterns of recognition for multiple compounds in herbal medicine[5-7]. Processed with chemometric technology, the entire pattern of compounds can then be evaluated to determine the identity and quality of herbal medicines and to ensure the consistency and stability of their related products. A big difficulty with chromatographic fingerprint analysis is the correction of retention time shift which is not solved very well yet now[8]. To avoid this difficulty, spectroscopic fingerprint analysis is applied to evaluate herbal medicine in this study. Several examples are presented in this paper to verify the validity of the method and the result of the experiment is satisfactory.

Theory and method: The fingerprint of herbal medicines is supposed to be a unique feature to determine the herbs, herbal extracts or preparations concerned. The construction of chemical fingerprints should be more useful for quality control of herbal medicines. It is well established that the samples with similar chemical fingerprints usually have the same physiological activities. The concept of phytoequivalence, which was developed by the Germans to ensure consistency and quality of the herbal products on the market[9], is the basic of the

fingerprint analysis. Therefore, the intuitive evaluation method is to compare the similarity and/or difference of the chemical fingerprints. The most widely used standards for evaluation of similarity of the multivariate systems are the correlation coefficient (r_{cor}) and the congruence coefficient (r_{con}), which are formulated respectively as follows[10]:

$$r_{cor} = \frac{\sum_{i=1}^{num}(x_i-\bar{x})(y_i-\bar{y})}{(\sum_{i=1}^{num}(x_i-\bar{x})^2)^{1/2}(\sum_{i=1}^{num}(y_i-\bar{y})^2)^{1/2}}$$

$$r_{con} = \frac{\sum_{i=1}^{num}x_iy_i}{(\sum_{i=1}^{num}(x_i)^2)^{1/2}(\sum_{i=1}^{num}(y_i)^2)^{1/2}}$$

where xi, yi are the ith elements in two different chemical fingerprints, say x and y, respectively and num is the number of the elements in the fingerprints. $\bar{x}$ and $\bar{y}$ are the average values of the num elements in fingerprints x and y, respectively, that is:

$$\bar{x} = \frac{\sum_{i=1}^{num}x_i}{num}; \quad \bar{y} = \frac{\sum_{i=1}^{num}y_i}{num}$$

If the data of chemical fingerprints have been standardized, the same result will be obtained by using either the indices r_{cor} or the indices r_{con}. Otherwise, the results of the two indices may be a little different. But, neither was shown to be always better than the other method. To avoid the effect of outliers, a median fingerprint can be used instead of an average fingerprint for similarity calculation. Here $\bar{x}$ and $\bar{y}$ are expressed by the other two formulas:

If num is odd, j is the median number from 1 to num,

$$\bar{x} = x_j; \ \bar{y} = y_j$$

If num is even, j and j+1 is the median two number from 1 to num,

$$\bar{x} = \frac{x_j + x_{j+1}}{2}$$

$$\bar{y} = \frac{y_j + y_{j+1}}{2}$$

using these formulae described above, one can calculate the similarity of a set of chemical fingerprints. The relationship within these fingerprints could be currently analyzed through comparing the similarity, presented as correlation coefficient or congruence coefficient. In order to illustrate the situation, the example of 16 herbal medicines is applied to address such a problem in this paper.

EXPERIMENTAL

Model FW80 (Taisite, Tianjin, China) all-purpose high-speed smashing machine was used for grinding the herbs. A commercial domestic microwave oven MDS22003F (Sineo, Shanghai, China) with a timer and variable power settings was used for microwave assisted digestion of the herbs. The digestion was carried out in DV-600 (Sineo, Shanghai, China) PTFE advanced composite vessel with 50 mL capacity. Maximum operating pressure was 3Mpa. Elemental analysis was carried out on Optima 4300DV inductively coupled plasma atomic emission spectrometer (PerkinElmer, Wellesley, USA). Table-1 shows the analytical lines used for each element, as well as the instrumental conditions.

TABLE-1
OPERATING PARAMETERS FOR ICP-AES

R_f frequency	27.12MHz		
Operating power	650W		
Coolant argon flow rate	7.51min^{-1}		
Plasma argon flow rate	0.81min^{-1}		
Burner type	minitorch		
Nebuliser type	meinhard		
Sample flow rate	2.3mL min^{-1}		
Detection wavelengths (nm^{-1})			
As	188.979	Mn	257.610
Ba	233.527	Mo	202.031
Ca	317.933	Ni	231.604
Cd	228.802	Pb	220.353
Cu	327.393	Se	196.026
La	408.672	Sr	407.771
Mg	285.213	Zn	206.200
Al	396.153	–	–

The water used in all studies was ultra-high-quality water (18MΩ cm) obtained from a Milli-Q system (Millipore, Milford, USA). All standard solutions used (1, 10, 100 and 1000 µg/L) were prepared by diluting 1 mg/mL stock multi-element standard solutions for ICP-AES (Perkin-Elmer, Wellesley, USA). For sample digestion, concentrated HNO_3 (NS, Nanjing, China) and 30 % (m/v) H_2O_2 (Shuanghuan, Beijing, China) were applied. During the experiments, all glassware and equipment were carefully cleaned starting with 2-4 % HNO_3 and ending with repeated rinsing distilled deionized water to prevent contamination.

Phytopharmaceutical samples: Samples of 16 herbal medicines were supplied as raw materials from local stores of Chinese traditional medicines in Shandong, China. The most widely accepted and most frequently consumed and commercially available brand names of herbal product were selected. To avoid purchasing adulterant herbal medicines, each of the samples has been authenticated by a local experienced herbalist. All the samples from the packages were ground into a fine powder and dried at 80 ºC for 24 h prior to use.

Analysis of the mineral content: The microwave digestion procedure was applied to herbal medicines samples. Dried samples of 0.500 g of the herbal medicines were placed in PTFE vessel for digestion. 6 mL of concentrated HNO_3 (65 %) and 2 mL of concentrated H_2O_2 (30 %) were used in microwave digestion system. After the digestion in microwave, the digest was transferred into a volumetric flask and made up to 50 mL with deionized water. A blank digest was carried out in the same way. Digestion conditions for the microwave system applied were: 3 min for 250 W, 3 min for 0 W, 5 min for 250 W, 5 min for 400 W, 6 min for 550 W, vent for 10 min.

Data analysis: For the chemometric study, each of the herbal medicines samples was characterized by 15 chemical descriptors, which are the contents of the analyzed elements. The software EXCEL2003 (Microsoft Corporation, Redmond, USA) was applied to plot a bar graph from the data matrix composed of 15 columns (the analyzed elements) and 16 rows

TABLE-2
CONTENT* OF METALLIC ELEMENTS IN THE HERBAL MEDICINES SAMPLES

Metal element	Jugeng	Chenpi	Kushen	Xiang jiapi	Houpu	Duzhong	Huaimi	Rougui
Al	0.436	0.326	1.065	2.362	0.639	1.484	0.498	0.123
As	0.037	0.000	0.000	0.017	0.000	0.000	0.000	0.000
Ba	0.054	0.310	0.157	0.321	0.516	0.542	0.038	0.774
Ca	1.450	3.461	8.100	7.804	4.174	4.667	3.706	8.653
Cd	0.000	0.000	0.000	0.000	0.004	0.006	0.000	0.002
Cu	0.058	0.039	0.053	0.094	0.088	0.068	0.158	0.054
La	0.001	0.003	0.005	0.005	0.003	0.004	0.002	0.002
Mg	34.390	2.779	4.607	6.716	3.176	4.394	7.313	1.503
Mn	0.079	0.126	0.143	0.443	2.232	0.780	0.247	1.208
Mo	0.000	0.002	0.008	0.004	0.003	0.004	0.013	0.000
Ni	0.005	0.007	0.009	0.015	0.011	0.010	0.025	0.000
Pb	0.022	0.027	0.033	0.016	0.232	0.164	0.029	0.054
Se	0.000	0.000	0.000	0.000	0.000	0.011	0.020	0.013
Sr	0.304	0.527	1.047	0.000	0.618	0.546	0.408	0.302
Zn	0.087	0.030	0.091	0.055	0.072	0.037	0.177	0.046
Metal element	Luhui	Qingteng	Chaihu	Jinyin hua	Bajiao huixiang	Yuxing cao	Yimucao	Pang dahai
Al	35.120	0.359	0.675	0.363	3.858	3.190	5.625	0.115
As	0.046	0.000	0.000	0.007	0.006	0.024	0.000	0.000
Ba	0.282	0.154	0.311	0.159	0.053	0.421	0.651	0.048
Ca	4.463	6.034	6.646	2.673	0.894	7.921	13.540	0.876
Cd	0.001	0.000	0.000	0.000	0.000	0.004	0.001	0.000
Cu	0.196	0.064	0.079	0.176	0.106	0.147	0.185	0.134
La	0.062	0.005	0.003	0.002	0.001	0.009	0.011	0.001
Mg	8.617	0.756	5.849	7.506	2.814	9.464	13.850	8.323
Mn	1.579	0.094	0.525	0.302	1.681	2.196	0.688	0.914
Mo	0.001	0.002	0.006	0.006	0.000	0.003	0.010	0.000
Ni	0.185	0.004	0.006	0.028	0.045	0.019	0.021	0.006
Pb	0.153	0.041	0.019	0.012	0.024	0.061	0.112	0.019
Se	0.000	0.003	0.000	0.013	0.020	0.040	0.000	0.015
Sr	1.726	0.918	0.646	0.668	0.089	0.791	1.135	0.150
Zn	0.132	0.087	0.065	0.154	0.100	0.169	0.173	0.202

*Mean of triplicate determinations.

(herbal medicines samples). Data analysis was performed on a Pentium 4 personal computer (CPU2.4GHz, RAM 512M). All programs for calculation were written in MATLAB 7.0 for Windows (The Mathworks, Natick, USA).

RESULTS AND DISCUSSION

Metal elements in herbal medicines: Metal elements are constitutive plant compounds with biological activity and are essential or toxic in metabolism. Much research has been made on metals to establish their normal concentration range and evaluate their role in herbal medicines as part of human medicinal treatment[11-13]. To construct fingerprints of herbal medicines, 15 metal elements in 16 samples are investigated. The metal content found in the study is shown in Table-2. The analytical results obtained for all metals indicate that they comply with the standard GB 2762-2005 which was enacted by the ministry of health of the People's Republic of China[14]. Looking at these values, Al and Ca and Mg are the elements with a major content in all samples, with average concentrations of 3.515 and 5.316 and 7.629 mg/L, respectively. Ba, Mn and Sr present lower contents, their average values 0.2994, 0.8272, 0.6172 mg/L. The other analyzed metals mostly appear with values lower than 0.1 mg/L. Macronutrient elements are needed in relatively large amounts in the human diet such as Ca and Mg. Several trace metals, such as Zn, Cu and Mn, are chemical elements that play an important role as oligoelements in several biological processes. However, these micronutrient elements may also be the origin of adverse effects on living organisms if the dosages exceed certain levels[15].

Fingerprint analysis of herbal medicines: As mentioned above, the analysis of one or more compounds as either active or 'markers' fails to be able to confirm the identity of a specific herbal medicine. The reason for this is that the markers or components are not unique to a particular herb. An active ingredient of a certain herbal medicine is often also found in many other herbs. The fingerprint of herbal medicines is considered to be a unique feature to identify the herb, herbal extract or preparation concerned. In fact, different kind of features can be selected to represent the herb medicine and used as 'fingerprint'. A rapid, simple and accurate analytical instrument is essentially required to obtain chemical fingerprints. In the present paper, the contents of 15 metallic elements have been determined in samples of herbal medicines by using ICP-AES. For convenience of recognition, the data obtained from the experiment was used to plot a bar graph (Fig. 1). Because the concentration and proportion of metallic elements differ between these herbal medicines, it is very easily found that each is presenting a unique fingerprint pattern. With the help of chemical fingerprints, one can accurately recognize a certain herbal medicine.

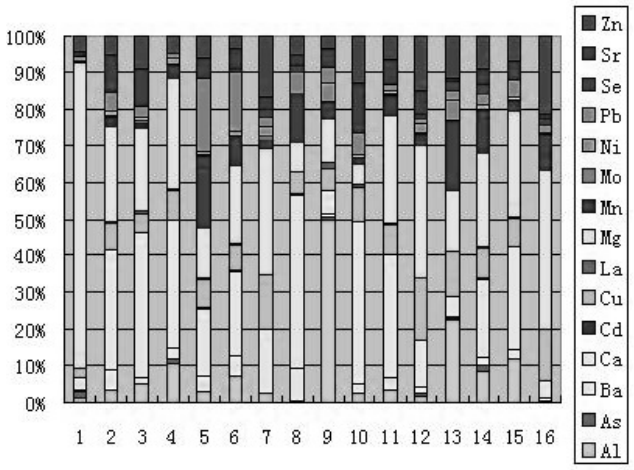

Fig. 1. Fingerprints of sixteen herbal medicines samples. 1 = Jugeng; 2 = Chenpi; 3 = Kushen; 4 = Xiangjiapi; 5 = Houpu; 6 = Duzhong; 7 = Huaimi; 8 = Rougui; 9 = Luhui; 10 = Qingteng; 11 = Chaihu; 12 = Jinyinhua; 13 = Bajiaohuixiang; 14 = Yuxingcao; 15 = Yimucao; 16 = Pangdahai.

Assessing the consistency of herbal medicines: Chemometric techniques are very useful in building the fingerprints of herbal medicines. With the help of chemometric techniques, an objective measure of the similarity could be processed which is surely necessary for regulatory and documentary purpose[16]. In the study, the chemical fingerprints of 6 batches of Yuxingcao extract were evaluated by comparing the similarity to determine batch-to-batch consistency. The relative content of metallic element Mg was given a value of 1. The evaluation by computer calculation showed a high degree of similarity between the 6 batches of Yuxingcao analyzed, suggesting a standardized consistency in raw material quality, represented by a correlation coefficient and a congruence coefficient of higher than 0.997.

Conclusion

In this study, spectroscopic fingerprint is proposed as a strategy for quality control of herbal medicines instead of reported chromatographic fingerprint. The results indicated that spectroscopic fingerprint is an effective and reliable tool for the purpose of species authentication and consistency assessment of herbal medicines. Western and traditional medical treatments are very different in their theory and practice. Thus, this is not an easy exercise of applying modern technologies to the quality control of herbal medicines. The complex relationship between the chemical fingerprints and efficacy of the herbal medicines seems to be the most important aspect for the quality control of herbal medicines, but the current fingerprint researches have not taken it into account yet. The quality control of herbal medicines is still at its beginning stage and needs a long way to go.

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