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# A novel strategy to evaluate the quality of traditional Chinese medicine based on the correlation analysis of chemical fingerprint and biological effect

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ABSTRACT

A novel strategy was developed to assess the quality of TCM (traditional Chinese medicine) based on the correlation analysis of the chemical fingerprint and biological effect. Using *Rehmanniae glutinosa* (*RG*) to treat the kidney yin deficiency as an example, chemical fingerprints of 27 *RG* samples were analyzed by liquid chromatography–mass spectrometry (LC–MS), and urinary metabolic profiling of *RG* treatment of kidney yin deficiency in rats was explored by using LC–MS. A correlation analysis between the chemical fingerprints and efficacy evaluation was developed to identify quality marker components to assess TCM quality. Thirty-four variables in chemical fingerprints were successfully defined to have a close relationship with the efficacy of *RG*. The validation test with a new *RG* sample indicated that these efficacy-related components could be used to evaluate the integral quality of *RG* accurately. Compared with conventional chemical fingerprint methodology, not only is the proposed approach a powerful tool to identify efficacy-related components for the quality evaluation of TCM, but the approach can also be used to predict the therapeutic efficacy of TCMs.



## 1. Introduction

Traditional Chinese medicine (TCM) had been prescribed clinically for thousands of years. Currently, TCM is gaining increased attention around the world, due to its distinguished therapeutic effects with minimal or negligible side effects. The chemical quality of TCM is important for its clinical efficacy. However, the discrepancies caused by planting locations, harvest seasons and processing methods pose great challenges to the standardization of quality control and assurance of TCM. Presently, considerable attention has been paid to TCM quality control. In the 2010 edition of the Chinese Pharmacopoeia, TCM quality control is primarily conducted using conventional analytical methods that mainly focus on limited types of compounds. For example, the interesting marker compounds of *Rhizoma coptidis* (Huanglian) and *Phellodendron Cortex* (Huangbo) are alkaloid berberines in the context of quality assurance. Many other active compounds have not been selected as target compounds, even if they can play roles in promoting clinical efficacy [1]. Efficacies of *Rhizoma coptidis* and *Phellodendron Cortex* have obvious differences. *Rhizoma coptidis* mainly clears "heat" in the middle part of the body, while *Phellodendron Cortex* acts on the lower part. A single compound analysis could not differentiate *Rhizoma coptidis* from *Phellodendron Cortex*. Hence, a single compound analysis of TCM quality makes assessment of real TCM functions difficult.

In recent years, the simultaneous determination of multiple compounds in TCM and chromatographic fingerprint analysis for quality control has gained prominence. Shi et al. identified and simultaneously determined eight major ingredients to control the quality of *Radix Tinosporae* by high-performance liquid chromatography (HPLC) [2]. The fingerprints of the root of *Salvia miltiorrhiza* for quality control were developed by LC–MS [3] and TLC Scan [4]. However, certain characteristic components for the quality control and chromatographic fingerprint peaks often failed to correlate with the therapeutic efficacies of TCM. Zhu et al. determined the contents of quality marker compounds and assessed the efficacy of Liu Wei Di Huang Wan preparations from 11 different TCM factories on the treatment for kidney yin deficiency. The results indicated that these samples had obvious different efficacies, while the contents of marker compounds met the standards of *Rehmanniae glutinosa* (*RG*) according to the 2010 edition of the Chinese Pharmacopoeia. Based on chemical fingerprint and efficacy relationship [1,5], the authors identified four characteristic components for the quality control. Hou et al. successfully screened bioactive compounds in an alkaloidal extract of *Alstonia scholaris* leaves using a cell receptor agonist functional evaluation model

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**Table 1**
Samples of *Rehmanniae glutinosa* analyzed in the present study.

| Raw *Rehmanniae glutinosa* | | Processed *Rehmanniae glutinosa* | |
|---|---|---|---|
| Code no. | Herb's origin | Code no. | Herb's origin |
| $RH_1$ | Wen county, Henan province | $PH_1$ | Wen county, Henan province |
| $RH_2$[a] | Wen county, Henan province | $PH_2$[a] | Wen county, Henan province |
| $RH_3$ | Wen county, Henan province | $PH_3$ | Wen county, Henan province |
| $RH_4$ | Wen county, Henan province | $PH_4$ | Wen county, Henan province |
| $RH_5$ | Wen county, Henan province | $PH_5$ | Wen county, Henan province |
| $RH_6$[a] | Wen county, Henan province | $PH_6$[a] | Wen county, Henan province |
| $RH_7$[a] | Wuzhi county, Henan province | $PH_7$ | Wuzhi county, Henan province |
| $RS_1$ | Xiangfen county, Shanxi province | $PS_1$ | Xiangfen county, Shanxi province |
| $RS_2$ | Xiangfen county, Shanxi province | $PS_2$ | Xiangfen county, Shanxi province |
| $RS_3$[a] | Xiangfen county, Shanxi province | $PS_3$[a] | Xiangfen county, Shanxi province |
| $RS_4$[a] | Xiangfen county, Shanxi province | $PS_4$[b] | Xiangfen county, Shanxi province |
| $RS_5$ | Xiangfen county, Shanxi province | $PS_5$ | Xiangfen county, Shanxi province |
| $RS_6$ | Linfen city, Shanxi province | $PS_6$ | Linfen city, Shanxi province |
| $RS_7$ | Yuncheng city, Shanxi province | $PS_7$ | Yuncheng city, Shanxi province |

[a] The herb samples used for both chemical fingerprint experiment and biological effect experiment.
[b] The herb sample as a new *Rehmanniae glutinosa* sample only used for the quality verification test of the correlation results between chemical fingerprint and biological effect.
The other samples without the superscript letters were only used for chemical fingerprint experiment.

coupled with HPLC [6]. Assessment standards should consider the therapeutic efficacies of TCM treatments, and any compound relevant to TCM quality control should be linked to its efficacy [7].

However, the holistic efficacy of TCM, with its multiple components and multiple targets, has been evaluated with difficulty by a few clinical biomedical indices. As a systematic approach, metabolomics could aid in evaluating efficacies of TCM and exploring its complex mechanisms [8]. Recently, considerable research has shown that metabolomics can be applied for the assessment of therapeutic efficacy. Wang et al. successfully assessed the therapeutic activity of Liu Wei Di Huang Wan in a rat model of kidney yin deficiency using metabolomics [9]. Metabolomics has also been used to differentiate herbal medicine of different origins and to identify characteristic components. Li et al. developed a novel strategy with metabolomics to identify potential marker components in order to differentiate raw and processed *RG* [10]. Because both the TCM samples and the biological samples are amenable to metabolomics, linking the chemical fingerprints of TCM to the biological effect observed after TCM administration is a reasonable approach to devise a new TCM quality control method.

*Radix Rehmanniae* (Dihuang), a "top grade" herb widely used in traditional Chinese medicines, is the root of *RG Libosch*. There are three forms of *RG* used in TCM practice: the fresh *RG*; the raw *RG*, which is the dried root of *RG*; and the processed *RG*, which is obtained by steaming or braising raw *RG* with rice wine or water. In the Chinese Pharmacopoeia, components for controlling the quality of the raw and processed *RG* are catalpol and acteoside, respectively [1]. However, the association of the two components with the pharmaceutical function of *RG* has not been clarified. A new approach is needed to control the quality of *RG*.

In this study, using *RG* to treat kidney yin deficiency, a novel method was developed to control TCM quality based on correlation analysis of the chemical fingerprints of various *RGs* and the biological effect of treated rats by the corresponding *RG* using LC–MS coupled with multivariate statistical analysis.

## 2. Experimental

### 2.1. Chemicals, solvents and herbal materials

HPLC-grade formic acid was purchased from Sigma–Aldrich (St. Louis, MO, USA). HPLC-grade acetonitrile was purchased from Merck (Darmstadt, Germany). Distilled water was prepared using a Milli-Q system (Millipore, Bedford, MA, USA). Thyroxine tablets were manufactured by Shanghai Pharmaceutical Limited Corporation of Industrial United Holdings Great Wall (Shanghai, China). Reserpine injections (1 mg reserpine/1 ml solution) were obtained from Guangdong Bangmin Pharmaceutical Limited Corporation (Guangdong, China). 10 ml of reserpine injections was added to 1.6 g of the powder of the thyroxine tablets, and the mixture was dissolved to 100 ml with distilled water for the animal modeling of kidney yin deficiency. Authentic standards, including 3,5-cyclic monophosphate (3,5-cAMP), L-gulonic acid, methionine, tryptophan, xanthurenic acid and kynurenic acid, were obtained from Sigma–Aldrich (St. Louis, MO, USA). Acteoside, 5-hydroxymethyl-2-furfural (5-HMF), and 8-epiloganic acid were purchased from the National Institute for Control of Pharmaceutical and Biological Products (Beijing, China).

Raw *Radix Rehmanniae* was collected from Henan Province and Shanxi Province (Table 1). The processed *RG* was produced by steaming and drying the raw *RG* for at least five cycles (generally nine times). Each cycle included steaming with water for 6 h followed by drying at 50 °C for 12 h.

### 2.2. Preparation of RG extract

To prepare analytical samples for the chemical fingerprint study, 50 g of *RG* was dissolved in 8 times its volume of water, refluxed at 100 °C for 2 h and later filtered. The residue was added to 6 times its volume of water and refluxed at 100 °C for 2 h. After 2 h, the solution was filtered, and the combined filtrate was subsequently concentrated to 100 ml at 40 °C in a negative pressure system. Next, 2 ml of the solution was pipetted into a 10-ml volumetric flask, scaled to capacity with methanol and settled at room temperature for 24 h. After centrifugation for 5 min at 5000 rpm, the supernatant was filtered through a 0.22-μm nylon filter (Agilent Technologies, Germany) and stored at 4 °C.

According to the preparation method of *RG* samples for the chemical fingerprint study, the samples of 0.8 g *RG*/ml water were prepared for the rat treatment of metabolomics study and stored at 4 °C.

### 2.3. Animal model handling and urine sample collection

The male Wistar-derived rats (age, 6 weeks; bodyweight, 200 ± 20 g) were provided by the Good Laboratory Practice (GLP)