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Near-infrared spectroscopy for rapid and simultaneous determination of five main active components in rhubarb of different geographical origins and processing

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ABSTRACT

Rhubarb (Rhei Radix et Rhizoma) is a classic herbal laxative medicine in Europe and a very famous natural medicine in Asia, especially in China. In this study, near-infrared spectroscopy (NIRS) was first used for rapid and simultaneous analysis of five main active components (chrysophanol, aloe-emodin, rhein, emodin and physcion) in rhubarb of 6 geographical origins, processing and spurious samples. A total of 124 samples (73 raw, 40 processed and 11 spurious samples) were collected. With the reference values determined by HPLC, two calibration strategies, partial least squares (PLS) as a linear regression method and artificial neural networks (ANN) as a nonlinear regression method, were studied. For the PLS strategy, 11 spectral pre-processing methods, 5 spectral regions and different latent variables (LVs) were systematically compared, while 3 spectral pre-processing methods and 5 ANN algorithms were studied for the ANN strategy. The results indicated that PLS was more suitable for the analysis of chrysophanol, aloe-emodin, emodin and physcion, whereas ANN was better for rhein. For the optimal NIR models of chrysophanol, aloe-emodin, rhein, emodin and physcion, the correlation coefficients of the calibration set (R_{cal}) were 0.9916, 0.9762, 0.9839, 0.9794 and 0.9800, respectively; the correlation coefficients of the prediction set (R_{pre}) were 0.9888, 0.9359, 0.9410, 0.9805 and 0.9785, respectively; the root mean square error of validation (RMSEP) were 0.0402, 0.0197, 0.0593, 0.0133 and 0.0192, respectively. Subsequently, the optimal NIR models were used to study the effects of geographical regions and processing, and identify the spurious rhubarb. Collectively, NIRS may be a well-acceptable method for quality evaluation of rhubarb and other traditional Chinese medicine (TCM).

1. Introduction

In the past decades, natural medicine, especially traditional medicine, was attracting more and more attention all over the world. Rhubarb (Rhei Radix et Rhizoma) is a high value and very famous natural medicine. It is a classic herbal laxative medicine in Europe and a very common cathartic and promoter of gastric health in China, Korea, Japan and some other Asian countries. In China, rhubarb, well-known as 'Daihuang', had been adopted as TCM originated from *Shennong's Herbal Classic* which is the earliest Chinese pharmacopoeia in the Eastern Han period (24–220 CE) [1–4].

Official rhubarb is derived from the dry root and rhizome of three species from *Rheum* Genus of Polygonaceae Family, *e.g. Rheum palmatum* L., *Rheum tanguticum* Maxim. ex Balf. and *Rheum offcinale* Baill. Unofficial rhubarb is from several related species of *Rheum* Genus, such as *Rheum emodi* Wall., *Rheum franzenbachii* Münt., *Rheum wittrochii* Lundstr., and *Rheum hotaoense* C.Y. Cheng et C.T. Kao. With strong side effect and low clinical efficiency, unofficial rhubarb is widely recognized as spurious rhubarb which could not be used in clinical practice. Rhubarb is mainly distributed and cultivated in Tibet, Sichuan province, Qinghai province, Gansu province, Shaanxi province, Hubei province and Yunnan province of China [3–5].

Abbreviations: 1st D, (first derivative); 2nd D, (second derivative); ANN, (artificial neural networks); BP-MLP, (back-propagation multilayer perceptron); COE, (constant offset elimination); GFF, (generalized feed-forward); LVs, (latent variables); MMN, (min max normalization); MSC, (multiplicative scatter correction); MSE, (mean square error); NIRS, (near-infrared spectroscopy); PLS, (partial least squares); *R*, (correlation coefficient); R_{cal}, (correlation coefficient of the calibration set); R_{pre}, (correlation coefficient of prediction set); RBF, (radial basis function); RMSECV, (the root mean square error of cross-validation); RMSEP, (the root mean square error of validation); RN, (recurrent network); RPD, (the residual predictive deviation); S-G, (Savitzky-Golay smoothing); SLS, (straight line subtraction); TCM, (traditional Chinese medicine); TLRN, (time-lag recurrent network); VN, (vector normalization).

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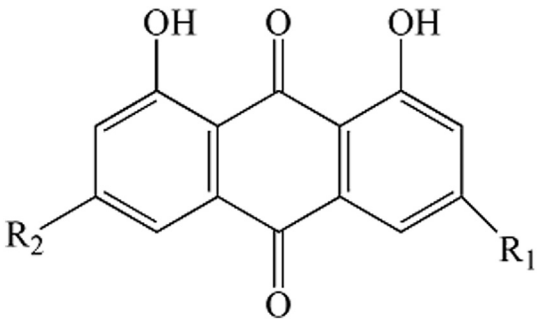
Researchers proved that rhubarb had the biological effect of purgative, antimicrobial, anti-inflammatory, antitumor, liver protective, and so forth [1, 3, 6]. The major pharmacological ingredients were anthraquinones and their glycosides, such as chrysophanol, aloe-emodin, rhein, emodin and physcion (chemical structures shown in Fig. 1). The above five components were widely considered as the main active components with various pharmacological actions, for example, antiviral activity of chrysophanol, cardiovascular protection and hepatoprotective activity of aloe-emodin, inhibitory on anaerobes and antitumorigenic effect of rhein, and antitumor, antimicrobial and antioxidant of emodin [1–3, 6, 7].

As well known, the clinical efficiency of TCM is depend significantly on its quality, which is affected largely by species, processing method, geographical origin and so on. As rhubarb had several species, geographical origins, processing methods and spurious samples, the quality of rhubarb must be controlled seriously to get good medicinal efficacy and clinical safety. The conventional methods for quality control of rhubarb were high-performance liquid chromatography (HPLC), capillary electrophoresis, supercritical fluid chromatography, *etc*. [2, 4, 6–8]. However, these traditional methods needed a long analysis time and required complex preparation procedures, so they could not be used for fast routine analysis and online analysis of rhubarb.

Generally, NIRS is the overtone and combination bands of the fundamental vibrations relating to CH, NH, OH and SH functional groups in the 12,000–4000 cm^{-1} wavelength range [9, 10]. With its characteristics of simple, fast and simultaneous analysis, recent studies had reported that NIRS had been successfully applied in the qualitative analyses and quantitative analyses of herbal medicine. However, the NIR spectra is characterized as broad peak, weak absorption and low selectivity. Therefore, NIR quantitative analysis need to use multivariate analysis methods, such as PLS, ANN, multiple linear regression, principal component analysis, ridge regression, support vector machines, *etc*. These approaches are mainly split into two groups: PLS is the most widely used method in linear regression group, while ANN is a remarkable method in non-linear regression group. [11–19]

Some researchers used NIRS for studying the quality of rhubarb [3, 5, 20–23]. However, these studies were limited to the qualitative analysis of official *VS* unofficial rhubarb [3, 5, 20–22] or quantitative analysis for total content of anthraquinones [23]. In this study, NIRS was first used for fast and simultaneous analysis the five active components (chrysophanol, aloe-emodin, rhein, emodin and physcion) in rhubarb of 6 geographical origins, processing and spurious samples. It could be emphasized that two calibration strategies, PLS as a linear regression method and ANN as a non-linear regression method, were studied and compared. Subsequently, the optimal NIR models were used to analyse the effects of processing and geographical regions, and identify the official and spurious rhubarb.



chrysophanol	$R_1 = CH_3$	$R_2 = H$
aloe-emodin	$R_1 = CH_2OH$	$R_2 = H$
rhein	$R_1 = COOH$	$R_2 = H$
emodin	$R_1 = OH$	$R_2 = CH_3$
physcion	$R_1 = CH_3$	$R_2 = OCH_3$

Fig. 1. Chemical structures of 5 main active components in rhubarb.

2. Materials and Methods

2.1. Samples and Reagents

In order to get a high degree of variability, a total of 124 rhubarb samples (73 raw, 40 processed and 11 spurious samples) were collected as shown in Table 1. To analysis the effect of regional variation, 72 raw samples were collected from 6 main geographical regions (Tibet, Qinghai, Gansu, Sichuan, Shaanxi, and Hubei in China). For study the processing effect, 6 batches, 2 batches from Gansu and Sichuan and 1 batch from Qinghai and Shaanxi, were processed based on the processing method of Chinese Pharmacopoeia (volume IV, 2015 edition). To identify the spurious samples, 11 unofficial rhubarb samples were obtained. The above dried samples were ground to pass through a 60-mesh sieve for HPLC and NIR analysis.

Standards of aloe-emodin and emodin (Batch No. 110795–201,609 and 110,756–201,512, respectively; purity >98% for both) were provided by the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, PR China). Chrysophanol, rhein and physcion (Batch No. BZP0046, BZP0043 and BZP0045, respectively; purity >97% for all) were purchased from Hefeibomei Biotechnology Co. Ltd. (Anhui, PR China). HPLC-grade methanol was from Tianjin Kermel Chemical Reagent Company (Tianjing, PR China). Water was purified by an ultrapure water instrument. All other solvents and reagents were of analytical grade unless otherwise noted.

2.2. Determination of Reference Values by HPLC

Rhubarb powder (Approximately 0.5 g, 60-mesh) was accurately weighed and extracted with 25 mL methanol. Each sample was extracted for 1 h by a heating reflux apparatus. Then, the sample was centrifuged at 12000 rpm for 3 min. At last, the extract solution was diluted with methanol into 25 mL and then filtered for HPLC analysis.

HPLC analysis was conducted on an Agilent 1100 HPLC system (Agilent Technologies Inc., CA, USA) equipped with a UV–Vis detector working at 254 nm. Every sample was separated on a Kromasil C_{18} column (4.6 mm × 250 mm, 5 μm) with column temperature at 35 °C. The mobile phase consisted of methanol/0.1% phosphoric acid (85/15, *v*/v) with a flow rate of 1 mL/min. The injection volume was 10 μL. The chromatographic peaks of chrysophanol, aloe-emodin, rhein, emodin and physcion (chemical structures shown in Fig. 1) were identified by spiking with the known standards and analyzed through external standard method. Table 2 and Table 3 listed the method validation data and the reference values of HPLC, respectively.

Above analysis method is referred to the current Chinese Pharmacopoeia (volume I, 2015 edition) which is the legal method for the quality control of rhubarb.

Table 1
Overview of a total of 124 samples.

	Raw sample	Processing sample on sale	Lab processed sample
Tibet	13	0	0
Qinghai	9	1	4
Gansu	12	1	12
Sichuan	36	1	12
Shaanxi	1	0	6
Hubei	1	0	0
Unknown	1	3	0
Spurious sample	5	0	6