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Rapid discrimination of sea buckthorn berries from different *H. rhamnoides* subspecies by multi-step IR spectroscopy coupled with multivariate data analysis

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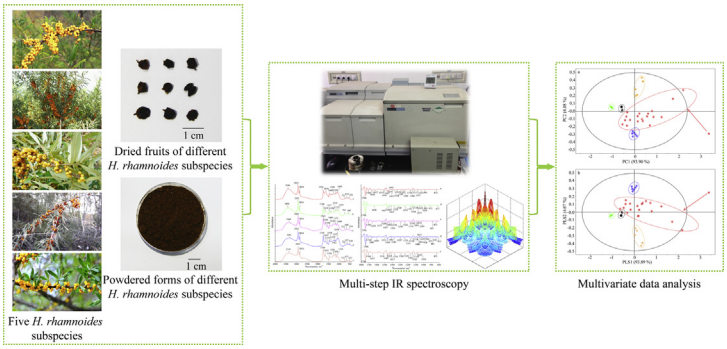
HIGHLIGHTS

- Multi-step IR spectroscopy was developed to identify five *H. rhamnoides* subspecies.
- Main components of sea buckthorn were characterized by multi-step IR fingerprints.
- Differences and similarities were revealed in IR spectra and chemometric analysis.
- Excellent sensitivity and specificity were showed in chemometric analysis.

GRAPHICAL ABSTRACT



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ABSTRACT

As an important ethnic medicine, sea buckthorn was widely used to prevent and treat various diseases due to its nutritional and medicinal properties. According to the Chinese Pharmacopoeia, sea buckthorn was originated from *H. rhamnoides*, which includes five subspecies distributed in China. Confusion and misidentification usually occurred due to their similar morphology, especially in dried and powdered forms. Additionally, these five subspecies have vital differences in quality and physiological efficacy. This paper focused on the quick classification and identification method of sea buckthorn berry powders from five *H. rhamnoides* subspecies using multi-step IR spectroscopy coupled with multivariate data analysis. The holistic chemical compositions revealed by the FT-IR spectra demonstrated that flavonoids, fatty acids and sugars were the main chemical components. Further, the differences in FT-IR spectra regarding their peaks, positions and intensities were used to identify *H. rhamnoides* subspecies samples. The discrimination was achieved using principal component analysis (PCA) and partial least square-discriminant analysis (PLS-DA). The results showed that the combination of multi-step IR spectroscopy and chemometric analysis offered a simple, fast and reliable method for the classification and identification of the sea buckthorn berry powders from different *H. rhamnoides* subspecies.

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1. Introduction

Sea buckthorn is a common name for all *Hippophae* species belonging to family Elaeagnaceae [1]. The ripe berry of sea buckthorn has been considered as a good source of large amounts of bioactive substances, such as vitamins, carotenoids, phytosterols, organic acids, fatty acids, free amino acids and different flavonoids [2,3]. They have a wide spectrum of medicinal and nutritional effects, including antioxidant, immunomodulatory, anti-atherogenic, anti-stress, hepatoprotective, radioprotective and tissue repair activities. For centuries, people in China have used sea buckthorn berries to prevent and treat various ailments, like asthma, skin diseases, gastric ulcers, mucous membranes stomach injuries and, cardiovascular diseases due to its nutritional and medicinal properties [3,4].

There are seven species and 11 subspecies belonging to *Hippophae* around the world, and seven species and seven subspecies in China, which are mainly distributed from the Hengduan Mountains to the Qinghai-Tibet Plateau [5–7]. The Chinese Pharmacopeia 2015 has admitted *H. rhamnoides* as botanical origins. [8]. According to previous scientific researches and the Survey on Chinese Materia Medica Resource, five *H. rhamnoides* subspecies, which are *H. rhamnoides* ssp. *sinensis*, *H. rhamnoides* ssp. *mongolica*, *H. rhamnoides* ssp. *yunnanensis*, *H. rhamnoides* ssp. *turkestanica* and *H. rhamnoides* ssp. *wolongensis* were distributed in China. Recent studies indicated that *H. rhamnoides* subspecies presented differences in their secondary metabolites spectra [4,9], which have led to a variation in treatment and health care efficacies [10,11]. Confusion and misidentification usually occurred because the same vernacular name "Shaji" was used for a number of different *Hippophae* species with similar morphology. Additionally, the authenticity of dried crude medicine was also difficult to ascertain. Therefore, it is essential to establish a rapid and accurate identification method to discriminate different origins of sea buckthorn, which would facilitate its quality control and guarantee the clinic therapeutic effect.

Fourier transform infrared (FT-IR) spectroscopy is a rapid and simple molecular spectroscopic method, for determining the holistic chemical characteristics and macro-fingerprint features. Second derivative infrared (SD-IR) spectroscopy provides enhanced resolution and indicates the overlapping information in the FT-IR spectra by amplifying minute differences. Two-dimensional correlation infrared spectroscopy (2DCOS-IR) can be employed to unfold FT-IR spectra in a second dimension to identify the differences and give more intuitive and remarkable information [12,13]. Multi-step IR spectroscopy, a technique consists of FT-IR spectroscopy, SD-IR spectroscopy and 2D-IR spectroscopy, has been widely used to analyze complex natural-product mixtures [14,15]. This approach can reflect both the holistic and detailed chemical characteristics. Therefore, the combination of multi-step IR spectroscopy and multivariate statistical analysis was successfully used as an effective method to discriminate and identify herbal medicines of different origins [16,17].

In this study, multi-step IR macro-fingerprinting has been employed for analyzing the main components and the holistic variation rules of their chemical constituents comprehensively. Moreover, coupled with multivariate statistical analysis, the present work also focuses on to develop a rapid, accurate and non-destructive method to identify sea buckthorn raw materials within the multi-botanical products of different origins.

2. Materials and methods

2.1. Apparatus

Spectrum GX Fourier-transformer infrared spectrometer (Perkin-Elmer Inc., Waltham, Massachusetts, USA), equipped with a mid-infrared deuterated triglycine sulfate detector (DTGS) was used. For each IR spectrum, 16 scans were performed at a resolution of 4 cm^{-1} in the range of 4000–400 cm^{-1}. The CKW-II portable and programmable temperature controller (Beijing Chaoyang Automatic Instrument Co., China) was applied within the temperature range of 50–120 °C [16,18].

2.2. Plant materials

In this study, a total of 41 berry samples of five *H. rhamnoides* subspecies were collected from the major producing areas in Sichuan, Qinghai, Tibet and Gansu, China (Table 1). All samples were identified by their morphological characteristics, and voucher specimens were deposited at the College of Ethnic Medicine, Chengdu University of Traditional Chinese Medicine, China.

2.3. Procedure

All the samples were oven-dried at 50 °C to completely eliminate moisture. Then, samples were pulverized into a fine powder and passed through a 200-mesh sieve. Approximately 1 mg of sea buckthorn berry powder per samples was mixed evenly with 100 mg KBr crystal. The mixture was grounded and pressed into a tablet using a 9.81 GPa pressure. After that, the 1D-IR spectra of all samples recorded from a total of 16 scans in the range of 4000–400 cm^{-1} with a resolution of 4 cm^{-1}. The interferences from H_2O and CO_2 were subtracted during the scanning process. The SD-IR spectra were converted from the smoothed and normalized 1D-IR spectra using the 13-point Savitzky Golay polynomial fitting method. Dynamic spectra were acquired at different temperatures, from 50 °C to 120 °C at an interval of 10 °C with a heating-up speed of 2 °C·min^{-1}. The 2D-IR correlation spectra were achieved by treating a series of temperature dependent dynamic spectra through the 2D-IR correlation analysis approach developed by Tsinghua University (Beijing, China). All analyses were repeated in triplicate, and all 1D-IR spectra were then converted to CSV file for further statistical analysis.

2.4. Data processing

With the aim of discriminating the sea buckthorn berries from different *H. rhamnoides* subspecies, principal component analysis (PCA) was used to give an intrinsic overview of the dataset and reveal possible groups and outliers [19]. Partial least squares discriminant analysis (PLS-DA) was also performed to maximize separation between groups [20]. Here, PCA and PLS-DA were conducted with mean-centered pre-process through SIMCA-P software (version 11.5, Umetrics, Umeå, Sweden).

3. Results and discussion

3.1. 1D-IR spectra characteristics of sea buckthorn berries from five H. rhamnoides subspecies

The 1D-IR spectra initially showed the general fingerprint characteristics of sea buckthorn berry powders from the five *H. rhamnoides* subspecies (Fig. 1). Each band represented the characteristic absorption peaks of functional groups in the sample. The strong and broad peak around 3400 cm^{-1} was corresponded to the —OH stretching vibration. Major peaks in the 3000–2800 cm^{-1} range were represented by the high intensity peaks at 2925 cm^{-1} and 2854 cm^{-1} belonging to the asymmetric and symmetric stretching vibrations of saturated C—H groups, while the stretching