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Analysis of herb–herb interaction when decocting together by using ultra-high-performance liquid chromatography–tandem mass spectrometry and fuzzy chemical identification strategy with poly-proportion design

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ABSTRACT

A novel and generally applicable approach was established for the herb–herb interaction analysis when decocting together by using ultra-high-performance liquid chromatography coupled with a triple quadrupole electrospray tandem mass spectrometer and fuzzy chemical identification with poly-proportion design. A simple programme was originally developed for the rapid identification and classification of herbal constituents on the basis of the establishment of herbal constituent databases, recognition of the reference compound peaks, selection of the diagnostic ions or fragmentation pathways, classification of chemical groups and formation of group networks. In this study, the exact structures of the chemical constituents did not need to be determined, and only the constituents attributed to different groups were further considered for quantitative analysis. Such a novel approach was successfully applied to kansui–licorice interaction analysis when decocting together. A total of 26 constituents from kansui and 45 constituents from licorice were classified into different chemical groups, and they were further quantitatively analyzed on the basis of semi-symmetric proportion design. The results showed that kansui could significantly promote the concentration of most triterpenoid saponins, phenylpropanoids and their glycosides (the constituents from licorice) in solution when co-decocting, and licorice could clearly promote the concentration of most diterpenes and triterpenes (the constituents from kansui) in solution, potentially explaining the incompatibility of kansui and licorice. Overall, the presently developed strategy should be useful for the interaction analysis for complex mixtures containing various complicated constituents, such as herbal, environmental, agricultural and biological samples.

1. Introduction

Traditional medicine—a system of ancient medical practices that differs in substance, methodology and philosophy from modern medicine—plays an important role in health maintenance for the peoples of Asia and is becoming more frequently used in Western countries [1–4]. Traditional medicine has formed its own distinct culture not only in China but also in other Asian countries like Japan, South Korea, Malaysia and Vietnam. For example, the traditional medicine in Japan is commonly called Kampo. In these different countries, different traditional medicines might use different prescriptions or methods of diagnosis, but the underlying philosophies and principles are similar because they all originate from China [5]. The composition of traditional Chinese medicine (TCM) is at odds with modern medicine. TCM uses formulae that contain several herbs to act in unison and restore what TCM practitioners call the patient's 'balance' [6]. As a unique medical system assisting the ancient and modern Chinese in dealing with disease, herbal combination therapies have been advocated for more than 2500 years in TCM [7]. However, Chinese medicinal herbs can have complex changes following their combination. Certain herbs reinforce or decrease their beneficial effects, while others moderate or eliminate their original toxic side-effects. Others can produce toxic

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and poor reactions, which can be attributed to variations of herbal bio-active constituents during *in vitro* decocting together and *in vivo* drug–drug interactions [8].

It is well known that studies on chemical interactions are extremely significant for disclosing the mechanisms of compatibility for medicinal herbs. Accordingly, the ability to perform qualitative and quantitative analyses of chemical component variations after medicinal herbs were decocting together has become a very important issue. However, the global detection and identification of the chemical constituents from herbs remain a great challenge because each herb contains many constituents that possibly belong to different structural types [9]. Although as many as dozens of components have been successfully identified from medicinal herbs or herbal preparations through the use of reference compounds and/or comparison with literature data [10–15], the reference compounds are always difficult to obtain, and many constituents in medicinal herbs are still unknown. Therefore, many researchers have also tried to develop other applicable approaches for the identification of a greater number of targeted [16–20] or non-targeted [21–24] components from herbal preparations using liquid chromatography coupled with mass spectrometry (LC/MS) analysis or other strategies, such as energy-gradient neutral loss screening and diagnostic ion filtering. However, it remains inevitable that without the corresponding reference compounds, these methods or strategies are unable to accurately identify all the multi-constituents, especially certain isomers in medicinal herbs, which prompted us to develop a better approach and strategy.

Due to improvements in chromatography [25], ultra-high-performance (or ultra-performance) liquid chromatography (UHPLC or UPLC) has been developed, which utilizes columns containing particles with a diameter of <2 μm and fluidic systems that operate at higher pressures [26]. When coupled with a tandem mass spectrometer, the system dramatically improves peak resolution, sensitivity and analysis speed [27–30]. Recent successes with the use of UHPLC or UHPLC–MS/MS for charactering and quantifying complex plant extracts [31,32], food [33,34] and metabolites in biological systems [35–40] suggest that the technique might also be effective for the comprehensively determining content variations of herbal multi-constituents after medicinal herbs are combined and decocted together.

In this study, our aim was to develop a generally applicable approach and methodology for the herb–herb interaction analysis when decocting together by using ultra-high-performance liquid chromatography coupled with a triple quadrupole electrospray tandem mass spectrometry (UHPLC/TQ-MS) and a fuzzy chemical identification strategy with poly-proportion design (Fig. 1). Such a strategy was predominantly inspired by an idea that most components in each herb can usually be classified into one of various chemical structure types, and the same type of component might connect with other components by diagnostic ions or fragmentation pathways produced in tandem mass spectrometry. Following this idea, a strategy involving the establishment of herbal constituent databases, recognition of peaks from the reference compounds, selection of the diagnostic ions or fragmentation pathways, classification of various chemical groups and formation of group networks was developed in the present study. With the benefit of self-established constituent databases, the MS data of reference compounds, and especially with the aid of the diagnostic ions or fragmentation pathways, the main peaks in the total ion chromatograms (TICs) of herbs A and B could be attributed to various chemical groups. In this study, the exact structures of the chemical constituents were not determined, and only the constituents attributed to the different groups were further considered for quantitative analysis based on the poly-proportion design for herbs A and B. Subsequently, the concentration variation curves of the constituents were constructed with gradually changing compatibility proportions [41], and the herb–herb interactions when decocting together were analyzed on the basis of these curves (Fig. 1).

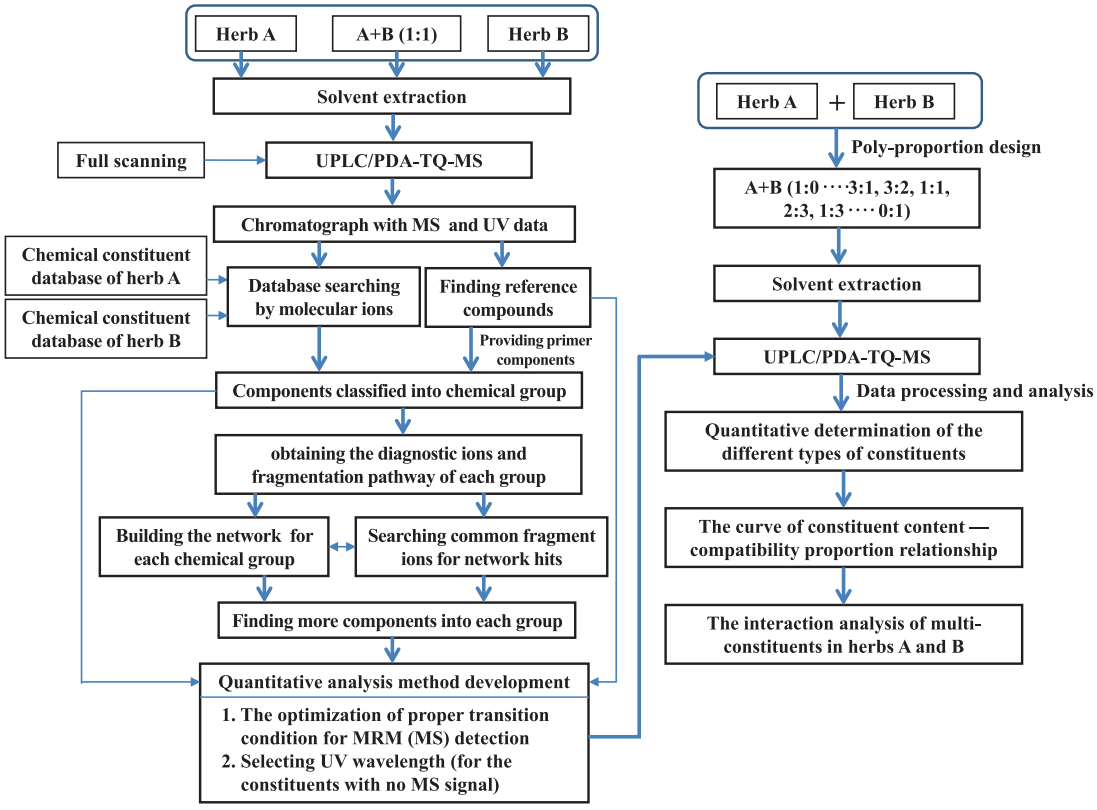

Fig. 1. Summary diagram of developed strategy and approach.