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Ultra-high performance liquid chromatography coupled with quadrupole/time of flight mass spectrometry based chemical profiling approach for the holistic quality control of complex Kang-Jing formula preparations

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ABSTRACT

The Kang-Jing (KJ) formula is a compound preparation made from 12 kinds of herbs. So far, four different methods (M1-M4) have been documented for KJ preparation, but the influence of preparation methods on the holistic quality of KJ have remained unknown. In this study, a strategy was proposed to investigate the influence of different preparation methods on the holistic quality of KJ using ultra-high performance liquid chromatography coupled with quadrupole/time of flight mass spectrometry (UHPLC-QTOF-MS/MS) based chemical profiling. A total of 101 compounds mainly belonging to flavonoids, tanshinones, monoterpene glycosides, triterpenoid saponins, alkaloids, phenolic acids and volatile oils, were identified. Among these compounds, glaucine was detected only in M3/M4 samples, while two dehydrocorydaline isomers merely detected in M2/M3/M4 samples. Tetrahydrocolumbamine, ethylic lithospermic acid, salvianolic acid E and rosmarimic acid were only detected in M1/M3/M4 samples. In the subsequent quantitative analysis, 12 major compounds were determined by UHPLC-MS/MS. The proposed method was validated with respect to linearity, accuracy, precision and recovery. It was found that the contents of marker compounds varied significantly in samples prepared by different methods. These results demonstrated that preparation method does significantly affect the holistic quality of KJ. UHPLC-QTOF-MS/MS based chemical profiling approach is efficient and reliable for comprehensive quality evaluation of KJ. Collectively, this study provide the chemical evidence for revealing the material basis of KJ, and establish a simple and accurate chemical profiling method for its quality control.

1. Introduction

Traditional Chinese Medicine (TCM), especially complex herbal medicine (CHM), is getting more and more attention because of its exact clinical efficacy and safety. Generally, traditional CHM is composed of multi-herbs and contain complicated components responsible for their efficacies. For one traditional CHM, there may be different methods documented for its preparation, and different preparation methods would affect the holistic quality of CHM [1]. However, the influence of different preparation methods on the holistic quality consistency of CHM lacks sufficient research and needs to be deeply studied. For CHM, especially those complex preparations containing more than ten herbs, their quality evaluation has always been challengeable using the approaches applicable to the analysis of a single plant TCM or simple TCM preparations. It is believed that multiple components, rather than single or several compounds, act on multiple-targets or interact synergistically with different biochemical pathways to perform the overall therapeutic functions of CHM preparations [2]. Therefore, it is critically important to establish an adequate and comprehensive analytical

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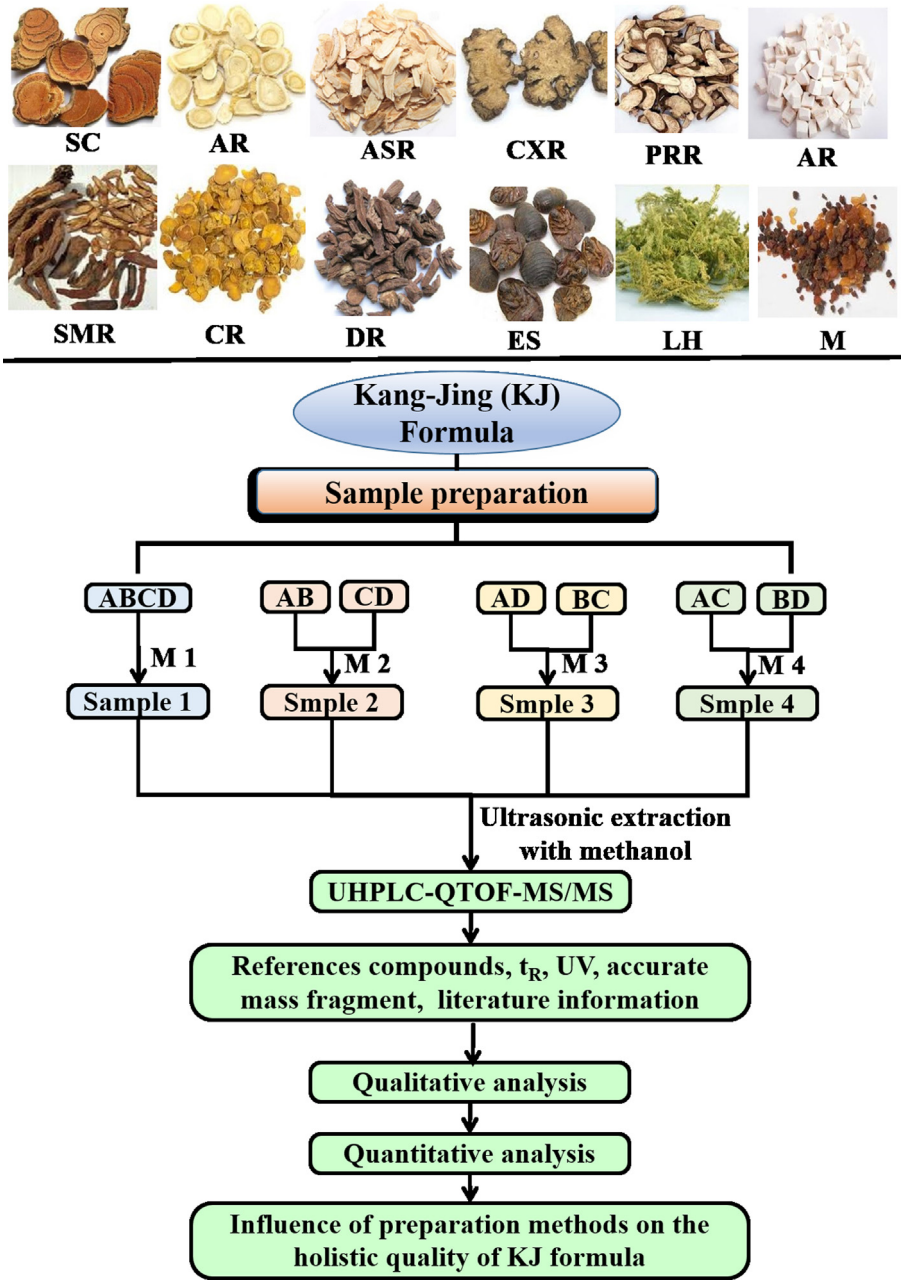

Fig. 1. Strategy proposed for exploring influence of preparation method on the holistic quality of KJ formula by UHPLC-QTOF–MS/MS based chemical profiling approach. A: DR (Dipsaci Radix), AR (Astragali Radix), PRR (Paeoniae Radix Rubra), and SMR (Salviae Miltiorrhizae Radix); B: ES (Eupolyphaga Steleophaga), LH (Lycopodii Herba) and CR (Corydalis Rhizoma); C: SC (Spatholobi Caulis) and PLR (Puerariae Lobatae Radix); D: ASR (Angelicae Sinensis Radix), CXR (Chuanxiong Rhizoma) and M (Myrrh).

method for evaluation of the holistic quality of traditional CHM to ensure their safety, efficacy, and batch-to-batch consistency.

The Kang-Jing (KJ) formula is a traditional complex herbal medicine made from 12 kinds of herbs, namely Spatholobi Caulis (SC), Dipsaci Radix (DR), Puerariae Lobatae Radix (PLR), Angelicae Sinensis Radix (ASR), Chuanxiong Rhizoma (CXR), Myrrha (M), Astragali Radix (AR), Paeoniae Radix Rubra (PRR), Salviae Miltiorrhizae Radix (SMR), Eupolyphaga Steleophaga (ES), Lycopodii Herba (LH) and Corydalis Rhizoma (CR). According to TCM theory, KJ possesses the activities of replenishing qi, dredging collaterals, activating blood flow to relieve pain, and is usually prescribed for treatment of cervical radiculopathy. In previous studies, it was reported that terpenes and saponins were the main components of PRR [3], DR [4], AR [5] and SMR [6], and nitrogen compounds were the main components of CR [7], LH [8] and ES [9]. The main components of PLR [10] and SC [11] were flavonoids. For ASR [12], CXR [13] and M [14], volatile oil were the major ingredients in these herbs. In present study, 12 herbs of KJ were divided into four types according to the principle components of every herb, such as type A of terpene and its glycosides compounds including PRR, DR, AR and SMR; type B of nitrogen compounds including CR, LH and ES; type C of flavonoids compounds including PLR and SC; type D of volatile compounds including ASR, CXR and M. The traditional preparation of KJ formula is that all herbs are mixed together for aqua extraction. Because 12 herbs of KJ can be divided into four types according to the principle components of every herb. To investigate the impact of preparation methods and interactions among different constituents on the holistic quality of KJ, the four types of herbs were mixed differently for aqua extraction during preparation process and were compared. Thus, four different preparation