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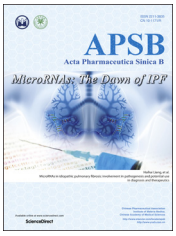

ORIGINAL ARTICLE

Identification and differentiation of *Panax ginseng*, *Panax quinquefolium*, and *Panax notoginseng* by monitoring multiple diagnostic chemical markers

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Panax species;
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Abstract To differentiate traditional Chinese medicines (TCM) derived from congeneric species in TCM compound preparations is usually challenging. The roots of *Panax ginseng* (PG), *Panax quinquefolium* (PQ) and *Panax notoginseng* (PN) are used as popular TCM. They contain similar triterpenoid saponins (ginsenosides) as the major bioactive constituents. Thus far, only a few chemical markers have been discovered to differentiate these three species. Herein we present a multiple marker detection approach to effectively differentiate the three *Panax* species, and to identify them in compound preparations. Firstly, 85 batches of crude drug samples (including 32 PG, 30 PQ, and 23 PN) were analyzed by monitoring 40 major ginsenosides in the extracted ion chromatograms (EICs) using a validated LC–MS fingerprinting method. Secondly, the samples were clustered into different groups by pattern recognition chemometric approaches using PLS-DA and OPLS-DA models, and 17 diagnostic chemical markers were discovered. Aside from the previously known Rf and p-F_{11}, ginsenoside Rs_1 could be a new marker to differentiate PG from PQ. Finally, the above multiple chemical markers were used to identify the *Panax* species in 60 batches of TCM compound preparations.

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1. Introduction

The roots of *Panax ginseng* (PG), *Panax quinquefolium* (PQ), and *Panax notoginseng* (PN) are used as the popular traditional Chinese medicine (TCM) Ren-Shen, Xiyang-Shen, and San-Qi, respectively[1]. Chemical compositions of the three species are very similar. Nonetheless, PG, PQ and PN are considered to possess different properties in TCM theory and thus exhibit different therapeutic functions. PG has the "warm" property and is a good invigorator; PQ is "cool" and is thus capable of heat-clearing and refreshing;[2] PN is mainly used to dispel stasis and stop bleeding. These functional varieties may originate from the difference in chemical composition, particularly in the bioactive triterpenoid saponins, popularly known as ginsenosides[1]. However, chemical difference among the three *Panax* species has not been fully clarified thus far. In addition, the market prices differ remarkably among the *Panax* species (for instance, between PG and PQ), and among the same species of different production areas (for instance, PQ cultivated in China and North America). Taken together, there is great demand to establish a reliable analytical method to differentiate the *Panax* species, and to identify their raw materials in TCM compound preparations.

Many analytical approaches have been used to identify *Panax* species, including DNA barcoding[3], Raman or infrared spectrophotometry[4–6], NMR spectroscopy[7,8], and LC–MS[9–11]. Among these approaches, LC–MS appears to be the most promising one. Wang et al.[10] reported the potential significance of two pairs of ginsenosides (Rg_1/Rf and Rc/Rb_2) in the differentiation between PG and PQ by LC/MS/MS analysis. Chan et al.[11] later reported the chemical markers ginsenoside Rf and 24(*R*)-pseudoginsenoside F_{11} together with the intensity ratio of ginsenosides Rg_1/Re for species differentiation of PG and PQ. However, a limited number of markers may not be able to fully depict the chemical differences between the three species. The results could be more definitive by monitoring multiple markers.

LC–MS-based fingerprinting followed by chemometric analysis has been increasingly used for TCM analysis, which enables species differentiation of congeneric plant species[12]. Direct infusion mass spectrometry combined with chemometric analysis has been reported to differentiate *Panax* species[13,14]. Our previous study has revealed the potential taxonomic significance of certain ginsenosides (oleanolic acid type, octillol type, malonylated, and peroxidized ginsenosides) in differentiating PG, PQ, and PN[15]. In this work, we present a new approach which integrates LC–MS based fingerprinting and pattern recognition chemometrics to discover more marker ginsenosides to differentiate these three species. These markers were further used in the identification of PG, PQ, and PN in 60 batches of TCM compound preparations.

2. Materials and methods

2.1. Chemical reagents and reference standards

Ginsenosides Ro, Ra_2, Ra_3, Rb_1, Rb_2, Rc, Rd, Re, Rg_1, Rg_2, Rf, 20-*O*-glc-Rf, and notoginsenosides R_1, R_2, R_4 were isolated from the roots of PG by the authors. Their structures were fully identified by NMR analysis[15]. 20(*S*)-Ginsenosides Rg_3, Rb_3, and 24(*R*)-pseudoginsenoside F_{11} were purchased from Nanjing Zelang Medical Technology Co., Ltd. (Nanjing, China). Their structures are shown in Fig. 1. The purities were >95% by LC–MS analysis. HPLC grade ammonium acetate (Fluka, Sigma–Aldrich, Netherland), formic acid, methanol, acetonitrile (J.T. Baker, Phillipsburg, NJ, USA) and ultra-pure water prepared using a Milli-Q water purification system (Millipore, MA, USA) were used for HPLC analysis. Analytical grade methanol and *n*-butanol were purchased from Damao Chemical Reagent Factory (Tianjin, China). The OASIS HLB Cartridge SPE columns were from Waters Corporation (Milford, MA, USA).

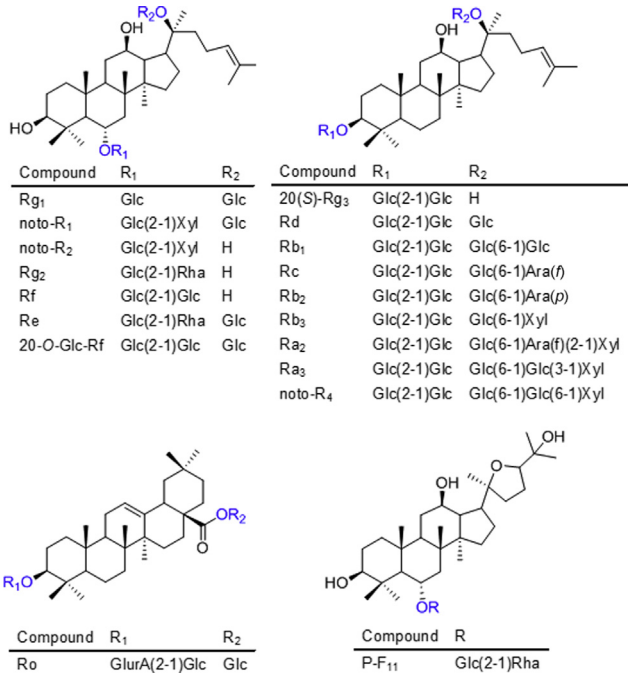

Figure 1 Structures for 18 ginsenoside reference compounds. Glc, *β*-D-glucopyranosyl; Rha, *α*-L-rhamnopyranosyl; Xyl, *β*-D-xylopyranosyl; Ara (*f*), *α*-L-arabinofuranosyl; Ara (*p*), *α*-L-arabinopyranosyl, GlurA, *β*-D-glucuronopyranosyl.

2.2. Plant materials

Crude drug samples of PG were collected from Northeast China or local Tong-ren-tang drugstores (Beijing, China). PN samples were collected from Wenshan County, Yunnan Province, China. PQ samples were purchased from Xushi Yangshen Specialty Co,. Ltd. (Nanjing, China). Detailed information for the 85 batches of samples is given in Supplementary Table 1. In addition, 60 batches of TCM compound preparations which contain PG, PQ or PN were purchased from local drugstores. Their information is given in Supplementary Table 2. Voucher specimens are deposited at the author's laboratory, School of Pharmaceutical Sciences, Peking University (Beijing, China).

2.3. LC–MS conditions

The LC–MS fingerprints were recorded on a Surveyor HPLC instrument coupled with a TSQ triple-quadruple tandem mass spectrometer *via* ESI interface (Thermo Fisher, San Jose, CA, USA). The samples were separated on a YMC-Pack ODS-A column (250 mm × 4.6 mm, 5 μm) equipped with an Agilent Zorbax SB-C18 guard column (12.5 mm × 4.6 mm, 5 μm). The column temperature was maintained at 35 °C. A three-component mobile phase was used, composed of acetonitrile (A), methanol (B), and water containing 1 mmol/L ammonium acetate (C). The