Development LC-MS/MS Method for Distinguishing between Natural and Artificial Compounds

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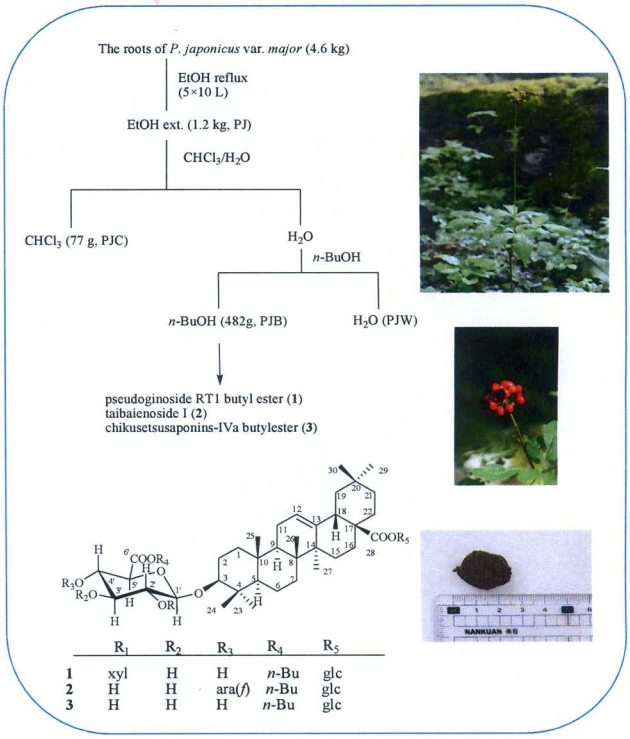
Abstract. *Panax japonicus* C. A. Meyer var. *major* (Chinese name, ZuTziSeng) is an important herb prescribed in traditional Chinese medicine to treat diabetes. In our study on the active constituents from its roots, we isolated three butyl oleanolic acid-saponins, pseudoginoside RT1 butyl ester (**1**), taibaienoside I (**2**) and chikusetsusaponins-IVa butylester (**3**). In order to confirm the three butyl compounds from natural plants rather than artifact compounds, they were analyzed by using LC-MS/MS with the optimized SRM parameters and the chromatograms. Furthermore, The ethanolic root extraction (PJ) and *n*-BuOH soluble portion (PJB) were also detected by the same way. In the results, PJ and PJB all exhibited the base peaks from three butyl compounds. Thus, the LC-MS/MS method could be applied to distinguish between natural and artifact compounds.

Introduction

The roots of *Panax* species (Araliaceae) are widely used in Chinese herbal medicine or food in Asian countries, because these plants are well-known for its medicinal properties. Pharmacological studies of triterpene saponins were isolated from *Panax* species and reported for anticancer, immunomodulatory, anti-inflammatory, anti-allergic, neuroprotective, antihypertensive, and anti-diabetic effects. The chemical constituents in *Panax* species are a group of triterpenoid saponins that can be classified into two groups based on their sapogenin skeleton, dammarane- and oleanane-type. *P. japonicus* C. A. Meyer var. *major* (Chinese name, ZuTziSeng) also contains many oleanolic acid-saponins and dammarane-saponins. It is an important herb prescribed in traditional Chinese medicine to treat diabetes (wasting-thirst), and exhibits expectorant, antitussive, hemostatic, sedative, and analgesic activities. As part of our investigation on the bioactive constituents of the roots of *P. japonicus* var. *major*, we isolated three butyl compounds and confirmed them as natural products by using LC-MS/MS method. This method was developed to distinguish between the minor natural and artifact compounds.

Results and Discussion

The dried roots of *P. japonicus* (4.6 kg) were pulverized and extracted with EtOH (5×10L) under reflux. The filtrate was concentrated under reduced pressure to obtain crude EtOH extract (1212 g, PJ). This extract was suspended into H_2O, and successively partitioned with $CHCl_3$ and *n*-BuOH to obtain $CHCl_3$-soluble brown syrup (77 g, PJC), *n*-BuOH soluble portion (482 g, PJB) and H_2O layer. The *n*-BuOH portion was chromatographed on a Dianion HP-20 column eluted with H_2O, followed by step gradients with MeOH to obtain seven fractions (Fr. 1–7). Pseudoginoside RT1 butyl ester (**1**), taibaienoside I (**2**) and chikusetsusaponins-IVa butylester (**3**) were further isolated from fr. 5 and 6 of PJB. In order to confirm the butyl compounds from natural plants rather than artifact compounds, compounds **1**-**3**, contained butyl group, were analyzed by using LC-MS/MS. Fragmentation of **2** produced ions of *m/z* 820 and 669 corresponding to $[M-H-Glc]^-$ and $[M-H-Glc-Ara]^-$, respectively (Figire 1A). In addition, the corresponding product ions of **1** and **3** also were shown in Figure 1B and 1C. The ethanolic root extraction (PJ) and *n*-BuOH soluble portion (PJB) were detected by using LC-MS/MS with the optimized SRM parameters and the chromatograms (Figure 2 and 3). SRM signals for *m/z* 981.6→651.4, 820→669 and 849.5→687.4 corresponding to **1**, **2** and **3** were detected simultaneously at 28.28, 26.30 and 29.32 min during LC-MS/MS, respectively.



Conclusion

The butyl compounds were confirmed in this plant, and the LC-MS/MS method could be applied to distinguish between natural and artifact compounds.

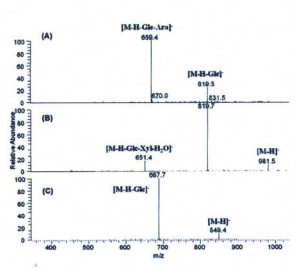


Figure 1. The product ion spectra of (A) taibaienoside I (**2**) (*m/z* 981.6), (B) pseudoginsenoside RT1 butylester (**1**) (*m/z* 981.6), and (C) chikusetsusaponins-IVa butylester (**3**) (*m/z* 849.5).

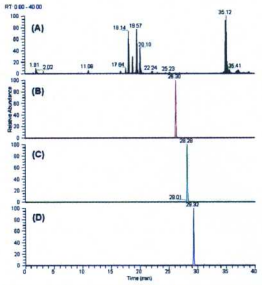


Figure 2. The base peak intensity (BPI) chromatogram (A) and the SRM chromatograms of (B) taibaienoside I (**2**) (*m/z* 981.6 > *m/z* 669.4), (C) pseudoginsenoside RT1 butylester (1) (*m/z* 981.6 > *m/z* 651.4), and (D) chikusetsusaponins-IVa butylester (**3**) (*m/z* 849.5 > *m/z* 687.4) for PJ.

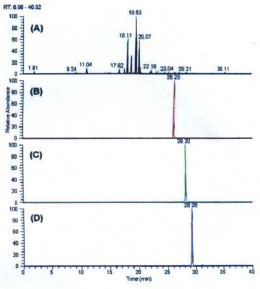


Figure 3. The base peak intensity (BPI) chromatogram (A) and the SRM chromatograms of (B) taibaienoside I (**3**) (m/z 981.6 > m/z 669.4), (C) pseudoginsenoside RT1 butylester (1) (m/z 981.6 > m/z 651.4), and (D) chikusetsusaponins-IVa butylester (**3**) (m/z 849.5 > m/z 687.5) for PJB.