Smashing Tissue Extraction and HPLC Determination of Paclitaxel and 10-Deacetylbaccatin from *Taxus x media*

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Abstract: **Objective** To optimize the extraction technology of *Taxus x media* by using the contents of Paclitaxel and 10-deacetylbaccatin (10-DAB), two representative active diterpene alkaloids of taxane type from *T. x media*, as evaluation standard. **Methods** The smashing tissue extraction (STE) of Paclitaxel and 10-DAB from *T. x media*, was investigated by comparing with ultrasonic extraction (UE) which was one of the modern technologies of extraction. **Results** STE was more efficient than UE, and the contents of 10-DAB and Paclitaxel in the extracts obtained by STE were higher than those by UE. **Conclusion** STE is a fast, high-performance, and energy-saving technology for the extraction of diterpene alkaloids of taxane type. STE also provides a simple, component-safe, workable, and highly efficient method for the extraction of active natural product.

Key words: 10-deacetylbaccatin; HPLC; Paclitaxel; smashing tissue extraction; *Taxus x media*

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Introduction

The taxanes is a general name of the diterpene alkaloid isolated from the plants in *Taxus* L. (Taxaceae) (Chen, Wei, and Yuan, 2001; Nair, 2003; Fang, 1994) and these compounds have been attracting more and more interest because of their strong anticancer effects. The extraction technologies of taxanes from the plants in *Taxus* L. have been investigated generously and the mainly reported technologies were Soxhlet extraction, solid phase extraction, ultrasonic extraction (UE), room temperature extraction, CO_2-supercritical fluid extraction, and enzyme-assisted extraction (Wang, Zhang, and Wang, 2007; Zhang *et al*, 1999; Wang, 2007; Man, Qiao, and Ni, 2008; Huang, Han, and Zhou, 2008; Zu *et al*, 2009). These extractions cost long time, large amount of solvents with poor selectivity, and more energy. Furthermore, the taxols, the major active compounds for anticancer, may easily cause change in the structure when heated for a long time. In this paper, the latest extraction technique called smashing tissue extraction (STE) was applied for the extraction of 10-deacetyl-baccatin (10-DAB) and Paclitaxel (Bentebibel *et al*, 2005) from *T. x madia* for the first time (Fig. 1). STE as a recently developed new technique has been successfully used for the extraction of polyphenols (Cao, 2009), phenolic acids (Zhang, Liu, and Li, 2007), saponins (Liu, Liu, and Zhao, 2009), and flavonoids (Deng, Song, and Cui, 2008) etc from various herbal materials. The basic principle of STE is to rely on mechanical shearing power and ultra-dynamic molecular filtration techniques to crush roots, stems, leaves, flowers, fruits, and other materials to crude powder within a few seconds in the presence of solvent at room temperature. To do so, the active ingredients rapidly reach the balance of internal and external tissues, and achieve the purpose of extraction by filtering. Because it took only about 3 min

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to finish an extraction, the proposed method was characterized as more convenient and faster than all traditional methods, and so it was called the flash extraction (Liu, 2007). It has been showing more potential as the most advanced technology in the extraction for the modernization of the Chinese materia medica (Wang and Zhao, 2009; Zhou, Liu, and Liu, 2009). We reported the extractive efficiency of 10-DAB and Paclitaxel (Fig. 1) from *T. x madia* by comparing with UE. Optimized extraction technology of taxanes was obtained with significant advantages for the first time.

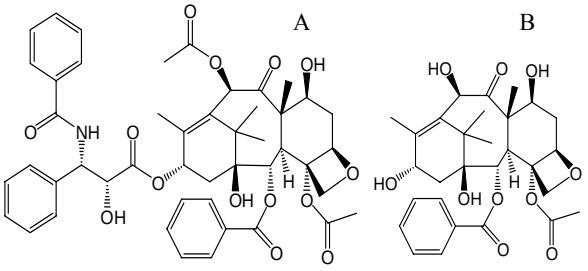

Fig. 1 Chemical structures of Paclitaxel (A) and 10-DAB (B) isolated from *T. x madia*

Materials and methods

Apparatus

The STE Apparatus JHBE—50S (Henan Jinnai Scien- Tech Development Co., Ltd., China); Sophisticated Electronic Balance BS—124S (Sartorius Company, Germany); Beijing Chuangwei high performance liquid chromatogram (UV3000 ultraviolet Detector and Attemperator), CXTH—3000 chromatography work station, and Kromasil C_{18} (250 mm × 4.6 mm, 5 μm) chromatographic column; Rotary Evaporator RE—52A (Sartorius Company, Germany); Thermostatic Water Bath Kettle (Taisite, China); Digital Ultrasonic Washer KQ3200DB (Kunshan Ultrasonic Instrument Co., Ltd., China).

Chemicals and reagents

The reference standards of Paclitaxel and 10-DAB were provided by Shenyang Biotechnology Limited Company; Alcohol was of analytical grade; Methanol and methyl cyanides were of HPLC grade; Water was purified water; The branches and leaves of *Taxus x madia* were supplied by Shenyang Biotechnology Limited Company and identified by Prof. SUN Qi-shi of Shenyang Pharmaceutical University.

Chromatographic conditions

Chromatographic analysis was performed with an Kromasil C_{18} chromatographic column (250 mm × 4.6 mm, 5 μm) and with a gradient mobile phase composed of acetonitrile (A) and water solution (B).

The following gradient was used: 0－35 min, maintain at 35%A; 35－60 min, linear gradient from 35% to 80%A; 60－70 min linear gradient from 80% to 35%A, maintain at 35%A until 80 min. The flow rate of mobile phase was 1.0 mL/min with UV detection at 227 nm. The operating temperature was maintained at 35 ℃ (Zheng, Ge, and Huang, 2004; Li *et al*, 2009).

Preparation of standard solution

A stock solution (1 mg/mL) of Paclitaxel (IS) was prepared by dissolving 10 mg of Paclitaxel with 10 mL of methanol in a volumetric flask. The standard solution of 1 mg/mL was obtained after shaking thoroughly. The solution (5 mL) was pipetted into 10 mL volumetric flask, diluted to the volume with methanol, and shaken well, and the working standard solution of 0.5 mg/mL was obtained. By further diluting the stock solution with methanol, a series of working standard solutions were obtained at the final concentration of 1.0, 0.5, 0.25, 0.125, and 0.0625 mg/mL, respectively. A stock solution (0.5 mg/mL) of 10-DAB (IS) was prepared by dissolving 5 mg of DAB with 10 mL of methanol in a volumetric flask. The standard solution of 0.5 mg/mL was obtained. By the same diluting method, a series of working standard solutions were obtained at the final concentration of 0.5, 0.25, 0.125, 0.062 5, and 0.031 25 mg/mL, respectively.

Preparation of sample

Ultrasonic extraction *T. x madia* (25 g) was sonicated with five times of 95% ethanol for 2 h at 45 ℃. Same procedures were repeated for another two times with totally 6 h. Combined ethanol extract was filtered and concentrated under reduced pressure. Finally, the residue dissolved in methanol was transferred to a 10 mL volumetric flask and diluted to the volume with methanol. The solution was filtered through 0.45 μm filter for HPLC analysis.

Smashing tissue extraction Branches and leaves of *T. x madia* (25 g) were extracted with five times of 95% ethanol by STE for 3 times. The first time is 3 min, the second 2 min, and the third 1 min. Six minutes in total were spent to finish the extraction. Combined ethanol extract was filtered and concentrated under reduced pressure. Finally, the residue was dissolved in methanol and transferred to a 10 mL volumetric flask and diluted to the volume with methanol. The solution was filtered

through 0.45 μm filter for HPLC analysis.

Results and discussions

Representative HPLC chromatograms of 10-DAB and Paclitaxel references substances and *T. x madia* were shown in Fig. 2. Corresponding peaks of 10-DAB and Paclitaxel could be easily recognized by comparing their t_R values.

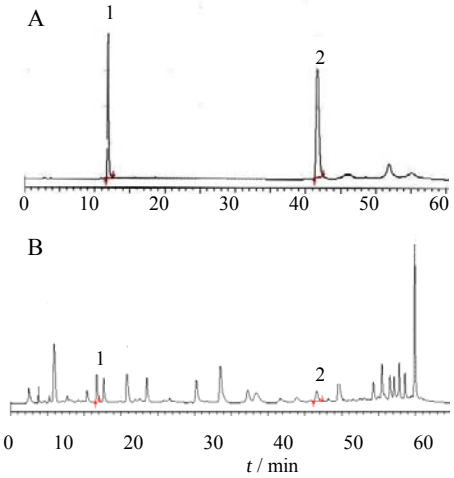

Fig. 2 HPLC chromatograms of Paclitaxel and 10-DAB reference substances (A) and STE sample of *T. x madia* (B)
1: 10-DAB 2: Paclitaxel

Calibration curves

Paclitaxel standard solution with different concentrations (10 μL) was injected into the HPLC injector and the responses were recorded. The standard curve (Fig. 3) was prepared by plotting the peak areas versus the quantity of Paclitaxel. The standard curve is $y = 2 \times 10^7 x + 298\ 125$, where $r = 0.9993$, $n = 5$, and r is correlation coefficient. It is concluded that a good linearity in the range of 0.000 625—0.01 mg was obtained.

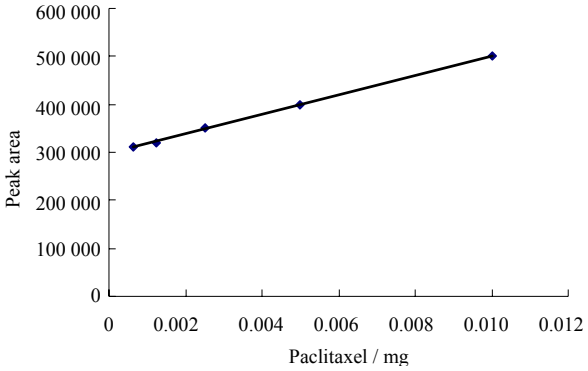

Fig. 3 Standard curve of Paclitaxel

10-DAB standard solution (10 μL) with different concentrations was injected into the HPLC and the responses were recorded. The standard curve (Fig. 4) was prepared by plotting the peak areas versus the quantity of 10-DAB. The regression equation is $y = 10^7 x + 32\ 857$ where $r = 0.9999$, $n = 5$ and r is correlation coefficient. It is concluded that a good linearity in the range of 0.000 312 5—0.005 mg was obtained.

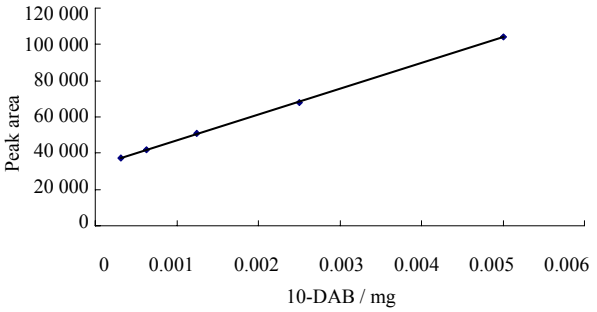

Fig. 4 Standard curve of 10-DAB

Precision

A total of six replicates of 10 μL STE samples prepared from the branches and leaves of *T. x madia* were performed under the same condition to determine the peak area repeatability of Paclitaxel. It is shown that the RSD of the repeatability is 0.32%. The result indicated that the instrumental precision was fine.

Stability

STE sample (10 μL) of the branches and leaves of *T. x madia* was performed under the same condition to determine the peak area of Paclitaxel after the solution was prepared immediately. And then the solution was determined repeatedly at 2, 4, 8, 12, and 24 h. RSD was calculated to be 0.67%. The result indicated that the stability of the sample solution was good within 24 h.

Repeatability

Six parallel tests were performed for STE sample prepared from 25 g of the branches and leaves of *T. x madia*. The solution (10 μL) was injected to HPLC to determine the peak area of Paclitaxel repeatedly. The average content of Paclitaxel was calculated to be 0.0231% with the RSD of 0.85%, indicating that the repeatability of the method was good.

Recovery test

The recovery test was determined by adding quantitatively reference substances to the sample solution of the branches and leaves of *T. x madia* with known amount of Paclitaxel. The mixture was analyzed and processed by using the methods of sample assay and the peak areas of Paclitaxel in different sample solutions were calculated. The results showed that the average recovery rate from five repeats was 98.4% with the RSD of 1.21%.

Quantitative determination

Sample solution (10 μL) was used to determine the content of Paclitaxel and 10-DAB under the condition mentioned above. By using the calibration curve the contents of Paclitaxel and 10-DAB were determined.

Conclusion

The contents of Paclitaxel and 10-DAB determined by STE from *T. x madia* were 0.0234% and 0.0330%, whereas by UE were 0.0215% and 0.0229%.

The results showed that the contents of Paclitaxel and 10-DAB from *T. x madia* by STE were slightly higher than that by UE. But it only took 6 min in total to finish the extraction, 1/60 comparing with UE. So, it is time-saving, energy-saving, and with high efficiency, *etc*. Another advantage is that STE is operated at room temperature, finished quickly, so as to avoid the possible decomposition or damage of taxols and other active components during the extraction process. It is demonstrated again that STE is the fastest, more efficient, safer, and greener new extraction technique. It could achieve better effect than UE to extract Paclitaxel and 10-DAB from the branches and leaves of *T. x madia*.

STE (Liu, 2007; Liu and Wang, 2010) has been successfully used for the extraction of different types of chemical components from Chinese herbal medicines. It is the first time to apply it for the extraction of diterpenoid Paclitaxel and 10-DAB from *T. x madia*. Its main advantages could be summarized as: 1) finished rapidly, processed efficiently, and extracted completely; 2) equipped simply, filtered easily; 3) operated at room temperature to avoid the decomposition or damage of heat-sensitive active ingredients; 4) applicable for various plant materials, such as roots (pieces), stems (pieces), leaves, flowers, and fruits, etc; 5) applicable for water, aqueous organic solvents, and most of organic solvents being depended on the solubility of specific components; 6) environment friendly: aqueous alcoholic solvent can be recovered easily, and the smashed residue can be easily recycled as many industry materials. On all accounts mentioned above, it is predicted that the industrialization of STE will lead the Chinese medicine industry to a new generation of technological innovation.

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