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Chemical profiles and metabolite study of raw and processed Polygoni Multiflori Radix in rats by UPLC-LTQ-Orbitrap MS^n spectrometry

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[ABSTRACT] The raw and processed roots of *Plygonum multiflorum* Thunb (PM) are used to treat different diseases in clinical practice. In order to clarify the influence of processing, a comparative study of chemical substance analysis was carried out. As the xenobiotics with a high enough exposure in target organs being considered as the potential effective or toxicity components, an *in vivo* study was also implemented to characterize the constitutes and metabolites, and meanwhile, the factor of compatibility with black bean were also considered. As a result, a total of 148 compounds were detected in PM extracts and more than 40 compounds were only detected in the processed products, which were probably new components produced during the steaming process. In *in vivo* study, 7 prototype components and 66 metabolites were detected or tentatively identified, 24 of which were reported for the first time. Our results indicated that processing greatly changed the chemical composition of PM and influenced the disposition of the compounds *in vivo*. To the best of our knowledge, this was the first global comparative study of raw and processed PM. These results expanded our knowledge about the influence of processing of PM and provided the essential data for further efficacy or toxicity studies.

[KEY WORDS] Polygoni Multiflori Radix; Processing; Chemical profiles; *In vivo* metabolite study

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Introduction

Chinese materia medica (CMM) processing, a unique pharmaceutical technology derived from the theory of traditional Chinese medicine (TCM), has played a prominent role in TCM clinical practice and ensured the safe and effective therapy for thousands of years. After processing, the appearance, properties, chemical composition, and pharmacological effects of each kind of TCMs have changed greatly, which is believed that processing could enhance the efficacy or reduce the toxicity of TCM.

Polygoni Multiflori Radix (*Heshouwu* in Chinese, PM) has been widely used in TCM clinical practice for centuries and is similarly popular in many other countries [1]. PM is commonly processed by steaming with black bean, rehmannia juice, or a wine- or ginger-black bean mixture, among which the black bean method is the most commonly used one and has been officially documented in the Chinese pharmacopoeia (Zhishouwu in Chinese, hereinafter calling "BPPM"). And PM steaming only with water is another common preparation method (hereinafter calling "SPPM") [2]. Lots of researches have shown that the raw root of *P. multiflorum* Thunb (RPM) and its processed products have different pharmacological effects. RPM mainly has purgative activity, including the efficacy of detoxication, eliminating carbuncle, malaria prevention and relaxing bowel. BPPM and SPPM are considered as tonic medicines for hair-blacking, liver-nourishing, kidney-nourishing, hematopoiesis, and so on [3]. In recent years, PM-induced liver toxicity has been attracted wide attention

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while processing could greatly reduce its toxicity [4-5]. In our previous studies, we have observed that black bean can reduce the hepatotoxicity of RPM [6-7]. In TCM clinical practice, the processed products are the more commonly used form, which are less toxic.

Up to now, most researchers have focused on the chemical component analysis of PM and more than 100 compounds have been isolated and identified [2], including stilbenes, anthraquinones, flavonoids, phospholipids, and phenolic acids as its main chemical constituents. Many studies have revealed that the stilbenes possess anti-oxidative, anti-aging, anti-tumor, anti-inflammatory and liver protective activities [8]. Anthraquinones have been proven to exhibit beneficial bioactivities such as antibacterial, antifungal, antiviral, antioxidant and anticancer effects [2]. Phenolic acid and flavonoid exhibit antioxidant activity *in vitro* and *in vivo*. In our previous study, we have also demonstrated that the contents of some chemical components are changed during the processing. The combined anthraquinones are hydrolyzed and the free anthraquinones, like emodin and physcion, are actually increased. Besides, the content of stibene glycosides is decreased gradually [9-12]. Other studies have found that processing results in the production of some new compounds, such as 2, 3-di-hydro-3, 5-dihydroxy-6-methyl-4(*H*)- pyran-4-one, hydroxymaltol, 5-hydroxym ethyl-furfural, butanedioic acid, and 5-dihydroxy-6-methyl- 4(*H*)-pyran-4-one [13-15]. Based on these reports, we considered that the substance change of chemical composition, resulting in different pharmacological effects and lower toxicity after processing, required a comprehensive and objective analysis.

In the present study, an effective and sensitive ultra-high performance liquid chromatography coupled with linear ion trap-Orbitrap MS^n (UHPLC-LTQ-Oribitrap) method was established and qualitative analysis of extracts of raw and processed PM *in vitro* were carried out to provide clear chemical profiles. Normally, only the exogenous chemicals with high enough exposure in target organs are considered as the potential effective and/or toxicity components. Therefore, RPM and its processed products were respectively oral administrated in rats and xenobiotics in rat biosamples were detected and characterized. To the best of our knowledge, this was the first global comparative study of raw and processed PM both *in vitro* and *in vivo*. It was hoped that these results would expand our knowledge about the influence of processing of PM, providing the essential data for its further efficacy and/or toxicity studies.

Materials and Methods

Materials

Standards of emodin and trans-2, 3, 5, 4′-tetrahydroxystilbene-2-*O*-*β*-D-glucopy-ranoside (THSG) were obtained from the National Institute for Food and Drug Control (Beijing, China). Gallic acid, catechin, epicatechin, emodin-8-glucopyranoside, and physcion-8-glucopyranoside were purchased from Shanghai Yuanye Biological Technology Co., Ltd. (Shanghai, China). Solvents were of HPLC grade and used without any further purification. HLB solid-phase extraction (SPE) cartridges (1 mL) were purchased from Waters (Milford, MA, USA).

The raw root of *Plygonum multiflorum* samples (No. 20160223) were purchased from Zhilv Wild Ecological Planting of TCM Cooperative (Guizhou, China). They were authenticated by Dr. HUANG Zhi-Hai and deposited at the Guangdong Provincial Hospital of Chinese Medicine.

Animals

Male, specific pathogen free Sprague–Dawley rats weighing 210–260 g were obtained from Guangdong Medical Laboratory Animal Center (Guangzhou, China) and housed in an environmentally controlled breeding room (temperature 20–26 °C, humidity 50%–70%) for 1 week and fed with standard laboratory food and tap water before experiments. The animals were fasted overnight, but supplied with water *ad libitum* before the experiments. The rats were executed by overdose of 10% chloral hydrate anesthetic. All the experimental protocols were approved by the Institutional Animal Ethics Committee of Guangdong Provincial Hospital of Chinese Medicine（2016.11.24, 2016035）.

Preparation of RPM, SPPM, and BPPM extracts

SPPM and BPPM were processed from the same batch of RPM in our laboratory. For the preparation of SPPM, the dry roots of RPM (1 kg) were infiltrated by water and steaming at 100 ºC for 24 h. The processed products were then dried under the drying oven at 55 ºC. For the preparation of BPPM, 100 g of black bean were extracted twice with water (2 × 600 mL, 4 h per extraction) and the combined extract was condensed to 200 mL. After infiltrated by the black bean extract, the roots of RPM were then steaming at 100 ºC for 24 h and then dried [16-17].

The dry roots of RPM, SPPM, and BPPM were extracted using following method: 1 kg dry roots were extracted thrice with 70% ethanol (3 × 800 mL, 60 min per extraction), and the combined extracts were condensed to 500 mL under reduced pressure, respectively.

Quantitative analysis of main compounds of PM extracts

The separation was performed by an Thermo Accela UHPLC instrument (Waltham, MA, USA) and the chromatography was carried out using a Thermo Hypersil GOLD C_{18} column (1.9 μm, 2.1mm × 50 mm) (Waltham, MA, USA). The mobile phase was composed of acetonitrile (A) and water containing 0.1% formic acid (B) using the following gradient program: 5% A (0 min), 10% A (2 min), 30% A (4 min), 30% A (7 min), and 60% A (10 min). The flow rate was set at 200 μL·min^{-1}.

The quantitative analysis was performed on a TSQ Quantum Ultra Triple Quadrupole LC–MS/MS system (Thermo Fisher Scientific, Bremen, Germany) using SRM mode. The contents of emodin, THSG, gallic acid, and catechin were calculated according to the established calibration curves. The SRM settings and contents of each compound are shown in Table 1.