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Paeonia veitchii seeds as a promising high potential by-product: Proximate composition, phytochemical components, bioactivity evaluation and potential applications

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ABSTRACT

To assess the application value of the seeds of the traditional medicine Chishao (*Radix Paeonia Rubra*, the root of *Paeonia veitchii* Lynch), the thousand seed weight, oil and kernel contents of the seeds, composition and contents of the main fatty acids, the physical and chemical characteristics of the seeds oil, and proximate composition and phytochemical components of the seeds shell and seeds oil residue were evaluated in this study. In addition, bioactivities such as antitumour and antimicrobial activity of the oligostilbene compound from *P. veitchii* seeds shell, and the inhibitory activities of the monoterpene glycoside compounds in *P. veitchii* seeds oil residue were also evaluated using different *in vitro* biochemical assays. The results showed that the seeds of *P. veitchii* had high oil content, and the seeds oil was characterized by very high levels of unsaturated fatty acids. The seeds shell contained large amounts of cellulose, lignin, and oligostilbene compounds, and high levels of monoterpene glycosides and crude protein were found in the *P. veitchii* seeds oil residue. The results of the biological tests showed that total oligostilbene compounds from *P. veitchii* seeds shell had antibacterial activity and moderate anticancer effects *in vitro*, and the total monoterpene glycoside compounds in *P. veitchii* showed strong inhibitory activities against NO production. As a medical plant and industrial crop with broad prospects, *P. veitchii* can be planted in most regions of China. In the past, it was planted solely because of the medical use of the roots. The above proximate and phytochemical profile, and bioactivity results show that the seeds of *P. veitchii* have high utilization value with potential in the development of value-added products, representing another valuable part of *P. veitchii* in the near future.

1. Introduction

Paeonia is a single genus in the paeoniaceae family, with three sections (*Moutan*, *Oneapia*, and *Paeonia*) and more than 35 species, which is widely distributed in temperate regions of the world (Hong and Pan, 1999; Wu et al., 2010). After thousands of years of breeding, cultivated *Moutan* and *Paeonia* have nearly more than one thousand strains derived from a few traditional strains with particular characteristics (Wang et al., 2004). *Paeonia veitchii* Lynch is the major Sect. *Paeonia* cultivar (Jia et al., 2014; Shi et al., 2016). The root of *P. veitchii* (*Radix Paeonia Rubra*, Chishao in Chinese) is a highly valuable Chinese Materia Medica, having been recorded in the Pharmacopoeia of the People's Republic of China (Shi et al., 2016; Yang et al., 2017). As a medical plant and industrial crop with broad prospects, the planting area of *P. veitchii* will increase in China because of the medicinal purposes of its root and the decrease of natural distribution. Additionally, the seeds are the most important by-product in these processes. The roots of *P. veitchii* is harvested every 4–5 years, while the seeds is obtained annually, which leads to large amounts of *P. veitchii* seeds each year (Li, 2016). Therefore, it is of interest to transform the current seeds waste into high value-added products.

Studies have shown that the seeds oil from some *Paeonia* species is rich in oleic acid, linoleic acid,α-linolenic acid and other unsaturated fatty acids with good nutritional value and health benefits, such as antithrombotic effects, significant inhibitory effects and hypotensive effects (Deng et al., 2010; Dong et al., 2013; Zhai et al., 2013; Zhu et al.,

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2010a,b; Liu et al., 2017c; Sevim et al., 2013), and the seeds oil from the kernel of *P. ostii* or *P. rockii* was authorized as a new food resource by the ministry of health of the People's Republic of China in March 2011 (Chen et al., 2016b). According to previously reported studies, the seeds of *P. ostii*, *P. rockii*, *P. suffruticosa* and *P. lactiflora* contain not only oil and protein but also many monoterpene glycosides, flavonoids, triterpenes, oligostilbenes and other secondary metabolites (Kim et al., 2002; Liu et al., 2013, 2014a,b, 2016a, 2017a,b, 2018a,b; Sarker et al., 1999; Wu et al., 2010; Yuk et al., 2013). As the same genus of *Paeonia*, the seeds of *P. veitchii* may also have important research and development value. Therefore, it is of great significance to evaluate the physico-chemical characteristics of the seeds from *P. veitchii*.

In this study, the physico-chemical characteristics of the seeds of *P. veitchii* was analysed, the proximate composition and phytochemical components of the seeds shell and seeds oil residue, the bioactivities of the secondary metabolites were determined, and the prospects for developing and utilizing the seeds of *P. veitchii* were evaluated. The aim of this work was to evaluate the comprehensive prospects of utilizing *P. veitchii* seeds and to provide scientific basis for this development and utilization.

2. Materials and methods

2.1. Plant materials

The seeds of *Paeonia veitchii* Lynch were collected from Zhongjiang County, Deyang, Sichuan Province, China in September 2017. The plant was identified by the expert Xin-sheng Wang (Professor of Chemical Engineering & Pharmaceutical College, Henan University of Science and Technology, Luoyang, Henan, China). The seeds were dried and then stored at 4 °C.

2.2. Chemicals

Fifteen pure monoterpene glycosides (Fig. 1) were isolated from an ethanol extract of seeds oil residue of *P. ostii* following methods described in the literature (Liu et al., 2018a). Seven pure oligostilbene compounds (Fig. 2) were isolated from an ethanol extract of seeds shell of oil peony following methods described in the literature (Liu et al., 2014a,b). The structures of the isolated compounds were confirmed using high-performance liquid chromatography (HPLC), mass spectrometry (MS), ^{1}H-NMR and ^{13}C-NMR, and their purity was determined to be more than 98% compared with the peak areas detected by HPLC-DAD (photodiode-detector).

HPLC-grade acetonitrile and methanol were purchased from Grace Company, Inc. (Houston, TX, USA). Acetic acid, phosphoric acid, and potassium phosphate dibasic were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Analytical-grade hexane, chloroform, methanol, ethyl acetate, petroleum ether, and others were purchased from Tianjin Kermel Chemical Reagent Co., Ltd. (Tianjin, China). The water was purified using a water purification system (Chengdu, China).

2.3. Characterization of the seeds of Paeonia veitchii

The seeds of *P. veitchii* were placed an oven and kept at 110 °C for 2 h, and the thousand seed weight (TSW) (g) of the seeds was calculated by the People's Republic of China national standard (GB/T 5519-88). The dried seeds were then shelled using a shelling machine (QTMP-1 peony seeds sheller, Lushan County Wantong Machinery Manufacturing Co., Ltd., Henan, China) to separate the seeds shell and seeds kernel. The kernel content (%) and shell content (%) were calculated according to the weight of the kernel and the shell compared with the weight of the seeds. The seeds kernel and seeds shell were ground into a powder with a cyclone mill (HCP-500A, Zhejiang province Yongkang city Jinsui Machinery Factory, Zhejiang, China) and then passed through a 40-mesh sieve. The seeds kernel and shell powders were collected and stored at 4 °C for future use.

2.4. Sample preparation

2.4.1. Seed oil preparation

The oil content in seeds of *P. veitchii* was determined according to the International Organization for standardization (ISO 659 1998). The oil was extracted from 5 g of the crushed seeds kernel sample over a period of 4 h with 100 mL *n*-hexane as the extraction solvent in a Soxhlet apparatus. The extraction procedure was repeated twice. The mixture of oil and the extraction solvent were separated *via* distillation in a Rotavapor (RE-2000A, Gongyi yuhua instrument co. LTD, Henan, China). The oil content of the seeds was calculated by dividing the mass of the extracted oil by the dry weight of the seeds. The seeds oil obtained was transferred into a glass vial and then stored in a freezer at −10 °C for further physical and chemical characterization determination. After extraction of the seeds oil, the seeds oil residue of the sample was collected, dried at 110 °C for 2 h, and weighed. The seeds oil residue content was calculated based on the weight of the seeds oil residue compared with the weight of *P. veitchii* seeds. The dried seeds oil residue was then stored at 4 °C for subsequent chemical constituent analysis.

2.4.2. Total monoterpene glycoside extract

The total monoterpene glycosides (TMGs) was extracted from seeds oil residue (prepared in 2.4.1) according to the method described in the literature (Liu et al., 2016a, 2018a). The powdered sample (5 g) was extracted in a 50-mL Soxhlet vessel with 100 mL methanol for 4 h, and the extraction solution was evaporated in a vacuum to dryness to obtain the TMGs. The TMGs was stored in a freezer at −10 °C for further total monoterpene glycoside and main monoterpene glycoside content determination.

2.4.3. Total oligostilbene compounds extract

The total oligostilbene compounds (TOC) extract in *P. veitchii* seeds shell was extracted using an ultrasound-assisted solvent extraction method (Liu et al., 2016b,c). Sample powder (5.0 g) was placed into a 50-mL tetrafluoroethylene extraction tube, which was filled with 40 mL ethanol and sonicated three times for 40 min at the 60 °C. The extraction solution was evaporated in a vacuum to obtain the TOC. The TOC was stored in a freezer at −10 °C for further oligostilbene compound content determination, and the antibacterial and antitumor activities were determined.

2.5. Characterization of the seeds oil of P. veitchii

The composition and content of fatty acids (FAs) in *P. veitchii* seeds oil prepared using the above method were determined according to the People's Republic of China national standard (GB/T 17376-2008 and GB/T 17377-2008). The seeds oil was first converted into fatty oil methyl ethers, and then the components were analysed with a GC–MS instrument (Liu et al., 2017c). The main FAs were determined by reference to corresponding authentic standards, and the relative amounts of individual components were calculated based on the GC peaks.

The physical and chemical characteristics of the seeds oil from *P. veitchii* were determined according to the International Organization for Standardization (ISO): peroxide value (POV) (ISO 3960, 2001), acid value (ISO 660, 1996), iodine value (ISO 3961, 1996), saponification value (ISO 3657, 2002), and moisture and volatile matter (ISO 662, 1998); and the People's Republic of China national standard for the determination of relative density (GB/T 5525-2008).