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Research paper

Comparing morphological, chemical and anti-diabetic characteristics of Puerariae Lobatae Radix and Puerariae Thomsonii Radix

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ABSTRACT

Ethnopharmacological relevance: Puerariae Lobatae Radix (PLR) and Puerariae Thomsonii Radix (PTR) are traditional Chinese medicines used for the treatment of diabetes and cardiovascular diseases. These two herbs are used interchangeably in clinical practice, even though they possess significantly different chemical profiles. In the case of *Pueraria* species, the misidentification is related to the multiple Chinese common names in clinical practice and variable pharmaceutical Latin names in different versions of the Pharmacopoeia of the People's Republic of China. In addition, there is lack of evidence demonstrating how the differences in the chemical profile would impact on the pharmacological activity of the two herbs. Therefore, the aim of this study was to compare the microscopic, phytochemical profiles and anti-diabetic activity of PLR and PTR so that the two species can be differentiated.

Materials and methods: The microscopic characteristics of the PLR and PTR were observed and measured by an optical microscope. The major compounds were quantified by ultra-performance liquid chromatography (UPLC) and total flavonoid content (TFC) colorimetric assay. The free radical scavenging capacity was assessed by 2,2-diphenyl-2-picrylhydrazyl (DPPH) antioxidant assays. Anti-diabetic activity was determined by the inhibition of porcine pancreatic α-amylase and rat intestinal α-glucosidase activities.

Results: Microscopic results illustrated that the size of xylem vessels (PLR: 0.1390 ± 0.0184 mm; PTR: 0.0471 ± 0.0109 mm), number of fibre per bundle (PLR: 32.6800 ± 2.8780; PTR: 16.5900 ± 0.9982) and the size of fibre (PLR: 0.0075 ± 0.0003 mm^2; PTR: 0.0025 ± 0.0002 mm^2) in PLR were significantly greater than that in PTR ($p < 0.01$). PLR possessed a significantly lower total starch content (PLR: 0.5288 ± 1.2559 mg starch/g DM; PTR: 76.7954 ± 2.9905 mg starch/g DM) and total dietary fibre content (PLR: 4.2886 ± 0.3466 g/100 g DM; PTR: 12.4148 ± 0.4541 g/100 g DM) as compared to PTR. Isoflavonoids including puerarin, daidzin, genistin and daidzein were the major chemical constituents in both species. However, the average content of puerarin in PLR was found to be eleven times greater than that in PTR. Furthermore, the TFC, DPPH free radical scavenging capacity, anti-α-amylase and anti-α-glucosidase in the PLR extracts were 4.42, 4.91, 3.10 and 4.22 times greater than in the PTR extracts.

Conclusions: This study provides a comprehensive investigation on the two medicinal valuable Pueraria species and allows differences to be ascertained. This information can be used to update monographs which will help practitioners and dispensers differentiate the herbs. Further study on the interchangeable use of PLR and PTR in clinical practice is urgently warranted.

1. Introduction

Correct species identification is crucial for ensuing the quality, safety and efficacy of a medicinal herb. The substitution and misidentification of medicinal herbs readily occurs in clinical practice, especially if they share similar morphological features or nomenclature, including analogous species names (Upton et al., 2011). In the case of *Pueraria* species, the misidentification is related to the multiple Chinese common names in clinical practice and variable pharmaceutical Latin names in different versions of the Pharmacopoeia of the People's Republic of China (PPRC).

Ge is a collective term used to describe the root of *Pueraria spp.* (Van der Maesen, 2002). It is one of the oldest medicinal herbs used in China and has been described in the Classic of Poetry dated between 1000 BC and 500 BC during the Zhou dynasty (Bodner and

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Hymowitz, 2002; Wong et al., 2011). Ge can be used to clear heat from fever, headache and stiffness in back and neck, nourish fluids by alleviating thirst caused by stomach heat, vent measles by accelerating rash at early stage and prevent spleen deficiency. The roots of *Pueraria lobata* (Wild.) Ohwi (Puerariae Lobatae Radix; PLR) and *Pueraria thomsonii* Benth. (Puerariae Thomsonii Radix; PTR) are commonly found in herbal pharmacies. Due to its long history and multiple dialects from different regions of China, multiple Chinese common names were used to describe these two herbs. For example, PLR is known as Ge Gen, Ye Ge, Gan Ge, Ye Ge Teng, Ge Hua Teng and Ge Tiao, whereas PTR is known as Fen Ge, Ge Shu, Gan Ge Teng and Ge Ma Ru (Bodner and Hymowitz, 2002; Van der Maesen, 2002).

The herbal industry and many traditional Chinese medicine dispensers/practitioners believe that these two species have similar efficacy and hence, use the two herbs interchangeable in manufacturing and clinical practice, respectively. Interestingly, PTR is a well-known ingredient in soups and other Chinese cuisines in South China which may allude to its food grade rather than medicinal application.

Initially, "Radix Puerariae" described both species in the first edition of the PPRC through to the seventh edition (2000). From the eighth edition, the herb was separated into two monographs namely, Radix Puerariae Lobatae and Radix Puerariae Thomsonii. In the latest edition of the PPRC (2010a,b), the Commission rearranged the order of the pharmaceutical Latin name, and they are now named Puerariae Lobatae Radix and Puerariae Thomsonii Radix.

Since the first inclusion of "Radix Puerariae" in the PPRC, the macroscopic and microscopic characteristics described in the pharmacopoeia have not been changed considerably. One of the major drawbacks of the current PPRC monographs is the lack of detailed descriptions and photographic illustrations, which make it difficult for users who are not familiar with pharmacognosy to distinguish the two herbs. In addition, morphological identification is rather subjective and the result may vary between different investigators and laboratories. Therefore, statistical analysis provides an objective comparison of the morphological characteristics. To provide quantitative data for the morphological characteristics, two colorimetric methods, which determine the total content of starch and dietary fibre, are employed herein (Englyst and Hudson, 1987; Rose et al., 1991). This approach is relatively fast and convenience compared to the preparation of conventional microscopic slides.

Apart from the morphological characteristics, chemical analyses such as high performance liquid chromatography (HPLC) and high performance thin-layer chromatography are recommended by the PPRC for the authentication of a medicinal herb (PPRC, 2010a,b). Polyphenols, in particular isoflavonoids, are the major chemical component in *Pueraria* species. Puerarin is the dominant chemical constituent and is used as the chemical marker to differentiate between PLR and PTR. Other common isoflavonoids identified in these two species include daidzin, daidzein, genistin and genistein (Wong et al., 2011). The chemical differences between PLR and PTR have been illustrated in a number of recent studies using various analytical instruments (Chen et al., 2006, 2014; Lau et al., 2009). However, these studies focused on the quantification of a single or a few individual chemical components and there is no investigation on whether such chemical differences would have an impact on their respective chemical and pharmacological activity.

In a previous study, the 2,2-diphenyl-1-picrylhydrazyl (DPPH) antioxidant capacity of PLR and PTR was assessed by HPLC coupled with flow injection chemiluminescence (Zhang et al., 2011). Hierarchical clustering analysis demonstrated that the DPPH-active chromatographic fingerprint of PLR was significantly different from PTR. However, this approach is time-consuming and requires special instruments, which restricts its application as a screening method. On the contrary, the adaptation of colorimetry with ELISA 96 well plate is rapid and convenient and hence, can be applied as a high-throughput screening method. Therefore, investigating the total flavonoid content (TFC) and DPPH antioxidant capacity of PLR and PTR using colorimetry could provide further insight on the chemical profiles of these two species.

PLR decoction has traditionally been used to treat and manage the symptoms in diabetic patients, however, the mode of action and chemical constituents that are responsible for such pharmacological activity remain inconclusive (Wong et al., 2011). The previous literature investigated the major chemical components from PLR in various *in vitro* and *in vivo* diabetic models, with only a handful of studies investigating the pharmacological activities of PLR extract and none on PTR extract. Puerarin, genistein and daidzein have been found to significantly decrease the baseline fasting plasma glucose, total cholesterol and insulin levels in several *in vivo* models (Hsu et al., 2003; Lee, 2006; Choi et al., 2008). Another therapeutic approach in the management of diabetes is the prevention of postprandial hyperglycaemia by inhibiting the activities of intestinal carbohydrate hydrolysis enzymes such as α-amylase and α-glucosidase (Bischoff, 1994). However, the pharmacological activity of PLR and PTR extracts on these two enzymes has not been investigated.

To the best of our knowledge, there is lack of comparative studies investigating the chemical and pharmacological differences of PLR and PTR. Furthermore, there is no evidence suggesting that differences in the chemical profile of these two species could impact on their respective pharmacological activities, thus supporting the clinical use of PTR as a medicinal herb. Therefore, the aim of this study was to provide comprehensive quality control data to distinguish between PLR and PTR. In this study, the morphological characteristics of the two species were compared. Subsequently, the phytochemical profiles of these two herbs were correlated to their antioxidant and pharmacological anti-diabetic activity. This study will provide extensive chemical data of the two *Pueraria* species and present new pharmacological actions on α-amylase and α-glucosidase activities. The outcomes of this study will emphasise the need for objective quality control procedures that will promote their correct application in clinical practice.

2. Methods and materials

2.1. Chemicals and solvents

Analytical grade methanol, chloroform, ethyl acetate, absolute ethanol (99.5% w/w), glacial acetic acid, acetone and HPLC-graded acetonitrile were purchased from Thermo Fisher Scientific (VIC, Australia). Puerarin (>98%), daidzin (>99%), daidzein (>99%), genistin (>98%) and genistein (>99%) were obtained from Tauto Biotech (Shanghai, China). Deionised water was purified by Siemens Ultra Clean Series water purification system (Siemens Water Technologies, NSW, Australia). All other chemicals and solvents were of analytical grade and were obtained from Sigma-Aldrich unless otherwise stated (NSW, Australia).

2.2. Plant materials

Forty-two dried samples (comprising of 22 PLR and 20 PTR samples) were purchased from herbal pharmacies in various regions of Australia, China and the United States of America. The collected samples were authenticated by Dr George Li (Faculty of Pharmacy, The University of Sydney, Australia). It is important to note that the 42 trade samples used in this study were identical as those used in our previous HPTLC and UPLC chromatographic fingerprint analysis studies (Wong et al., 2013, 2014). The 42 trade samples were authenticated by comparing the macroscopic, microscopic and chemical