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Original Article

# Pharmacognostical studies of *Premna microphylla*

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ABSTRACT

Various traditional systems of medicine enlightened the importance of *Premna microphylla* Turcz., Lamiaceae, medicinally. The present study was carried out to provide a scientific basis of the identification and the authenticity of *P. microphylla* with the help of pharmacognostical parameters, which is not done before. Roots, stems, and leaves of *P. microphylla* were collected for pharmacognostical studies involving macros, microscopic evaluation, physicochemical parameters analysis like fluorescence analysis and thin layer chromatography, in addition with DNA barcodes of internal transcribed spacer and *psb*A-*trn*H regions. Transverse section of root indicated the presence of stone cell bands. Transverse section of stem showed the presence of stone cells and vessels. Transverse section of leaf midrib revealed the presence of shaft type of porosity. Microscopic studies of powder revealed the presence of cork cells, fibers, vessels, nonglandular hairs, stone cells and glandular scale cells. Thin layer chromatography of the extract revealed the presence of oleanolic acid in *P. microphylla* with specific $R_f$ values. Identification through DNA barcode showed the sequence of internal transcribed spacer region was novel while the sequence of *psb*A-*trn*H region displayed no differences from known sequence. The observations confirmed that *P. microphylla* has an obvious pharmacognostical characteristics, which will be useful toward providing a reliable basis for identification, purity, quality and classification of the plant.



## Introduction

*Premna microphylla* Turcz., a medicinal and edible plant locally known as “doufuchai”, belonging to the family Lamiaceae, is a perennial deciduous shrub with a height of 2–6 m tall, which widely distributes in tropical and subtropical regions of the world such as Asia, Australia and Africa, and in China. It is broadly distributed in the mountainous regions in the east, middle, and south. Normally it grows up in acid soil environment where the altitude lies from sea level to 500–1000 m (Zhang et al., 2017). The leaves, blade is ovate-lanceolate, elliptic, ovate, or obovate in shape, measuring 3–13 cm long and 1.5–6 cm wide. The apex is long acuminate to acute, and based part is narrowly cuneate with margin entire or lobed to sometimes serrulate. The petiole ranges the length from 0.5 to 2 cm long. Inflorescences are in the form of conical panicles. Fruit is purple in color with globose to obovate in shape. The seeds, with 1000-grain weight about 17.5 g, are difficult to breed though the setting rate is high.

*Premna microphylla* has been highlighted the use of its roots, stems and leaves as Traditional Chinese Medicine (TCM) for the treatment of numerous ailments like skin burning and bleeding, rheumatism, dysentery, swelling and viper bites (Chen et al., 2014). While the antioxidant, antimicrobial and cytotoxic activities have been found in essential oil (Zhang et al., 2017), leaves and stems (Xu et al., 2010) from this plant. In addition, the extracts of its leaves which can be used to prepare “green tofu” by local people for its high amount of pectin, have been used to treat fatigue and inflammation (Chen et al., 2014).

The chemical composition and efficacy of extracts from *P. microphylla* were intensively investigated in previous studies. Two new xanthones (Wang and Xu, 2003), four new isoflavones (Zhong and Wang, 2002), a new triterpene glycoside (Zhan et al., 2009) as well as two new glyceroglycolipid and ceramide (Zhan and Yue, 2003) from the extracts of this plant have been isolated and their structures elucidated. In addition, fifty-six compounds were identified in the essential oil of *P. microphylla* (Zhang et al., 2017). The pectin from *P. microphylla* leaves has been extracted for analyzing the cell wall composition, observing the morphology of residues after each extraction steps and presenting physicochemical properties of different pectic substances (Chen et al., 2014). The complete nucleotide sequence of the *P. microphylla* chloroplast (cp) genome was reported and characterized (Yang and Kong, 2016). As for the pharmacognostical studies, the anatomic structure of the stems and leaves of *P. microphylla* have been reported (He et al., 2011), which was the only one report involving the pharmacognostical studies of it so far but it was not complete and systematic. Therefore, the

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information regarding its pharmacognostical identification on the roots, stems and leaves is still very scanty and poor understood.

As the drug is endowed with huge exploitation and utilization value, it is medicinally important to know precisely and comprehensively about its characteristics of pharmacognosy. With some supplementary in the previous study on plant anatomy of *P. microphylla* (He et al., 2011), herein we made a detailed investigation on macroscopic, microscopic characters, histochemistry, physicochemical parameters, fluorescence analysis, powder behavior and DNA barcodes of this plant to help in its identification and standardization.

## Materials and methods

### *Collection and identification of plant material*

Fresh plants of *Premna microphylla* Turcz., Lamiaceae, were collected from Guangdong Pharmaceutical University, Guangzhou Higher Education Mega Center (23°3′39″ N 113°23′54″ E), identified and authenticated by Prof. Shengguo Ji, School of Chinese Traditional Medicine, Guangdong Pharmaceutical University. They were washed and cleaned by flowing water to remove the physical impurities, air-dried in the shade, made into powder in a blender and preserved in hermetic container with dry air for pharmacognostical study.

### *Preparation of sample*

#### *Transverse section*

Transverse sections of fresh roots, stems and leaves of *P. microphylla* were made by hand, which were immobilized in FAA solution (formalin:glacial acetic acid:70% ethyl alcohol; [5:5:90]) for macro- and microscopic observations (Johansen, 1940).

#### *Leaf epidermis*

Fresh leaves of plant were subjected to obtain the upper and lower epidermis by tweezers, put into a petri dish with distilled water and cut into slices to be observed of cells and stoma. The stomatal index is calculated by the following formula (Kang, 2005).

$$\%\ \text{of Stomatal Indices} = \frac{\text{Stomatal number per unit area}}{\text{Stomatal number per unit area} \times \text{Epidermal cell number of same area}} \times 100\% \quad (1)$$

#### *Powders*

The powder was separately treated with glycerine (50%, v/v) and chloral hydrates (10%, v/v) through the heating for microscopic study.

### *Macroscopic characters*

The organoleptic and morphological characters of fresh material including color, shape, size, texture and fracture were studied and noted.

### *Microscopic characters*

The powders of plant were studied microscopically and fresh material fixed with the FAA was used for histologic study. Sliced by paraffin section method, the thin hand cut sections of roots, stems and leaves were dehydrated in a series alcohol concentration, followed by staining with safranine-fast green, mounting with neutral resin (Johansen, 1940). Microphotographs were taken by observing the free hand sections under Motic Multi-plexer attached to the microscope. All important features were detected and recorded suitably.

### *Physicochemical parameters analysis*

The physicochemical parameters analysis of the powders, including behavior of powder drug, fluorescence and TLC analysis were determined as per the standard guidelines (Kokate, 1998; WHO, 1998; Khandelwal, 2001).

#### *Behavior of powders*

The powders were treated with different regents namely glacial acetic acid, sulfuric acid, hydrochloric acid, nitric acid, ferric chloride, sodium hydroxide and potassium hydroxide. The behavior of powders like floating or sinking and changes of solution colors were observed.

#### *Fluorescence analysis*

Fluorescence analysis was carried out by ultrasonic processing the powdered drug with different reagents, namely ethanol (75% v/v), ethyl acetate, acetone, methanol, chloroform, carbon tetrachloride, water and petroleum ether and observed at 254 nm, 366 nm in a UV chamber and visible light.

### *Thin layer chromatography*

Thin layer chromatography studies were carried out for methanol extract of *P. microphylla* and reference sample oleanolic acid. The spots obtained from both the extracts were examined under visible light. An aluminum plate (20 cm × 10 cm) precoated with CMC-Na (0.5%)-silica gel G was used as the absorbent. The solvent system was toluene-ethyl acetate-glacial acetic acid (12:4:0.5). The methanol extract of *P. microphylla* was prepared by using 2 g powder, ultrasonic treated with 50 ml of methanol, filtered and evaporated to dryness. The plate was developed in a Camag twin trough chamber at temperature of 14 °C and relative humidity 69%, and sprayed with 10% ethanol sulfuric acid for coloration, dried at 105 °C and examined at visible light.

### *Identification through DNA barcode*

#### *Extraction of genomic DNA*

The samples were cleaned with pure water, followed by being scrubbed with ethanol (75%, v/v) and placed in a mortar adding liquid nitrogen to be quick-frozen, and made into coarse powder (Marieschi et al., 2012). The total DNA was extracted by Plant DNA Extraction Kit (Guangzhou Xueyou Biotechnology Co. Ltd., China, Batch number: 2017011537) following manufacturer's instruction, and its purity was checked using Ultraviolet Micro-Spectrophotometer (K5500Plus, Beijing Kaiao Technology Development Co. Ltd., China).

#### *Amplification of internal transcribed spacer region*

Complete internal transcribed spacer (ITS) region of *P. microphylla* was amplified with the universal primers ITS4 (forward primer; 5′-TCCTCCGCTTATTGATATGC-3′) and ITS5 (reverse primer; 5′-TCCTCCGCTTATTGATATGC-3′) (Balasubramani et al., 2011a). The primers were custom synthesized by Shenzhen Huada Gene Technology Co. LTD. (Shenzhen, China). Amplification was carried out in 20 μl reaction volume with 5.9–8.6 μl sterile double-distilled

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