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Original Article

Comparative authentication of *Semiliquidambar cathayensis* and its substituted species via macroscopic and microscopic features

Dan Zhu [a,1], Xincheng Qu [b,1], Xuemei Sun [a], Shihuan Yan [a], Hongwei Guo [a,*], Yaoli Li [c,*]

[a] Guangxi Key Laboratory for Bioactive Molecules Research and Evaluation & College of Pharmacy, Guangxi Medical University, 22 Shuangyong Road, Nanning 530021, China
[b] Guangxi Institute of Chinese Medicine & Pharmaceutical Science, Nanning 530022, China
[c] School of Pharmaceutical Sciences, Peking University, Beijing 100191, China

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ABSTRACT

Objective: Ban Fenghe recorded in the *Quality Standard of Yao Medicine of Guangxi Zhuang Autonomous Region* (Volume 1) is derived from the dried stems and leaves of *Semiliquidambar cathayensis*. It is usually confused with medicinal herbs from *Pterospermum heterophyllum* and *Dendropanax dentiger*. However, they are very different in chemical composition, and should not be used as the same drug. To ensure their safety and efficacy, a method based on macroscopic and microscopic characteristics was developed to distinguish them.

Methods: A total of 14 batches of Ban Fenghe samples from three species were collected from different producing areas in China. The macroscopic characteristics were examined by observing external traits. The tissue structures of transverse sections of stems and leaves, the leaf epidermis, and the powder were observed microscopically.

Results: The branchlets and leaf surfaces of *S. cathayensis* and *P. heterophyllum* were hairy, especially the lower leaf surfaces of *P. heterophyllum* were densely covered with hairs, but those of *D. dentiger* were hairless. The pericyclic fibers of *S. cathayensis* stems were intermittently distributed in a circular shape and accompanied by stone cells, whereas those of *P. heterophyllum* and *D. dentiger* were bundled without stone cells. So stone cells and hairs were present in *S. cathayensis* powder, stone cells were not found in *P. heterophyllum* and *D. dentiger* powder, and hairs were not present in *D. dentiger* powder. The distribution sites, sizes and types of secretory tissues of these three species were also different in transverse sections of stems and leaves. Stomata on the lower epidermis of *S. cathayensis* leaves were paracytic, whereas those of *P. heterophyllum* and *D. dentiger* were anomocytic.

Conclusion: Ban Fenghe drugs derived from *S. cathayensis* could readily be distinguished from those of *P. heterophyllum* and *D. dentiger* by macroscopic and microscopic features.

1. Introduction

Semiliquidambar cathayensis H. T. Chang, which is called "Ban Fenghe", "Jin Lv Ban Fenghe", and "Ban Hefeng" in Chinese, is a plant species of the Hamamelidaceae, mainly distributing in Guangxi Zhuang Autonomous Regions, Guangdong, Jiangxi Provinces in China. Its dried stems and leaves, showing anti-rheumatic, activating blood and dispersing blood stasis effects, are used as Yao medicine to treat rheumatoid arthritis, lumbar muscle strain, and bruises (Xu et al., 2017) by local people of Guangxi for many years, and recorded in *the Quality Standard of Yao Medicine in Guangxi Zhuang Autonomous Region* (Volume 1) (Food and drug administration of Guangxi Zhuang Autonomous Region, 2014).

However, more than 20 medicinal herbs from eight different genera of six plant families have similar names as *S. cathayensis*, most of them named "Ban Fenghe" in Chinese, but they have different efficacies (Zhang, Yun, Niu, Lin, & Wang, 2008). According to the literatures and our surveys on Guangxi markets, the most commonly used drugs are dried stems and leaves derived from *Pterospermum heterophyllum* Hance (Sterculiaceae) and *Dendropanax dentiger* (Harms) Merr. (Araliaceae) (Yang et al., 2016). *P. heterophyllum* is known as "Ban Fenghe", "Banbian Fenghe", or "Yinyang Ye" because the leaves on its young trees and newly germinated branches are split like maple leaves, whereas the other leaves are

* Corresponding authors.
E-mail addresses: hongweiguo@gxmu.edu.cn (H. Guo), liyaoli123456@163.com (Y. Li).
[1] These authors contributed equally to this work.

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full and like lotus leaves. It was first reported in the Lingnan Collection (*Lingnan Caiyao Lu*), and can be used to treat rheumatoid arthritis (Nanjing University of Chinese Medicine, 1999). *D. dentiger* is also known as "Shu Shen", "Feng Hegui", or "Ban Fenghe" (Guangxi Institute of Botany, 2005), and its leaves are changeable, with both entire and lobed leaves often coexisting on the same branch. Its roots, stems and leaves can be used to treat migraines, rheumatic arthralgia, etc. Therefore, these three drugs are often confused due to their similar morphologies, Chinese names and efficacies.

These three medicinal herbs have been studied in chemical composition and pharmacological effects (Chien et al., 2014; Lu et al., 2015; Wang et al., 2012; Wei, Chen, Ye, & Zhou, 2012). The ethyl acetate soluble fraction of the rhizome of *S. cathayensis* shows significant anti-inflammatory activity, and was found to contain mainly oleanolic acid derivatives and ellagic acid derivatives (Zhou, Yang, Shi, & Yang, 2002). The chemical constituents contained in the ethyl acetate soluble fraction of *P. heterophyllum* roots are flavanol and flavone (Wei, Chen, Ye, & Zhou, 2012). The extract of *D. dentiger* roots exhibits anti-inflammatory effect, and its major constituents are phenol derivatives (Yang, Liu, Fan, Zhong, & He, 2021). These results show that they are similar in the pharmacological effects but very different in chemical composition (Yang, Wang, Liu, & He, 2016). Therefore, the chemical basis and mechanism of anti-rheumatism effect of these three medicinal herbs are different. Moreover, according to *the Quality Standard of Yao Medicine in Guangxi Zhuang Autonomous Region* (Volume 1), there is only one source of "Ban Fenghe", which is the dried stems and leaves of *S. cathayensis*. Therefore, they should not be used as the same drug. However, only a few studies focused on their identification (Lin & Lou, 2010; Tian, Zeng, Yan, Jiang, & Xiang, 2018; Yang, Yao, Shu, & Sheng, 1996; Yuan, Li, & Liu, 2009), and it is difficult to distinguish them, especially when the leaves and stems are broken or powdered. Therefore, it is necessary to develop an efficient and reliable method to authenticate and distinguish the three medicinal herbs for the sake of safety, efficacy, and quality control.

Macroscopic and microscopic identification are traditional methods for authentication of traditional Chinese medicines. Due to its rapid, economical and environment-friendly advantages, it is widely used up to now (Gul et al., 2019; Kandemir, Celik, Ullah, Shah, & Zaman, 2019; Li, Shang, Chen, & Cai, 2013; Ur Rahman et al., 2019; Yu et al., 2014; Yuan, Liu, Wang, & Zhang, 2019). To identify the drugs derived from *S. cathayensis* accurately, we used macroscopic and microscopic identification techniques to analyze and compared the features of the three medicinal herbs, so as to provide scientific evidence to distinguish the three species of Ban Fenghe and also a basis for the quality control and clinical use of the Ban Fenghe drug from *S. cathayensis*.

2. Material and methods

2.1. Materials

Samples of *S. cathayensis*, *P. heterophyllum*, and *D. dentiger* were collected from the major production regions in China (Table 1) and authenticated by Yunfeng Huang (Associate Research Fellow at Guangxi Institute of Chinese Medicine & Pharmaceutical Science). The voucher specimens were deposited in the Pharmacognosy Laboratory, College of Pharmacy, Guangxi Medical University.

2.2. Apparatus

Transverse sections from the three species of Ban Fenghe were prepared using a semiautomatic slicer microtome (Leica RM2245, Germany). A light microscope (Olympus BH-2, Japan) equipped with a digital camera (Olympus DP20, Japan) was used to acquire photographs.

2.3. Reagents

Chloral hydrate and diluted glycerin solvents were prepared according to the procedures described in Part four of the Pharmacopoeia of the People's Republic of China (2020). Formaldehyde–acetic acid–ethanol (FAA) solution was obtained by mixing 70% ethanol, 38% formaldehyde, and glacial acetic acid in a 90:5:5 ratio and was used to fix the specimens. Tissue materials were dehydrated using a graded ethanol series (from 30% to 100%). Safranin and Fast Green solutions were prepared for specimen staining. Paraffin wax and turpentine were also used in this study. The chemical reagents used in the experiment were of analytical grade.

2.4. Macroscopic and microscopic examination

The macroscopic characteristics of the stems and leaves from *S. cathayensis*, *P. heterophyllum*, and *D. dentiger* were examined by observing their external traits, such as shape, size, color, surface characteristics, texture, and cross-sections, and this information was recorded. Stem samples (7–10 mm in length, 3–6 mm in diameter) were taken from branchlets. To assess the consistency of microscopic characteristics, three different locations (bottom, middle and top) were sampled for each sample, and ten slides for each location of each sample were prepared. The materials were fixed in FAA solution for 24 h, processed and embedded in paraffin, then sectioned by microtome to obtain the transverse stem sections (Attar, Esfandani-Bozchaloyi, Mirtadzadini, Ullah, & Zaman, 2019; Yu et al., 2017; Zhao et al., 2017). The fixed and treated leaves were cut into 5 mm × 10 mm pieces along the midrib to obtain paraffin transverse sections of the leaf. The leaf transverse sections were stained with safranin and Fast Green. Microscopic specimens of the leaf surface and powder were prepared according to the method described in the Chinese Pharmacopoeia (Volume 4, 2020 Edition) (National Pharmacopoeia Commission, 2020). The tissue structures of the transverse stem and leaf sections, the epidermis of the leaf, and the medicinal powder were observed microscopically. The features were photographed and described upon observation.

3. Results

3.1. Macroscopic morphological characteristics

Photographs of the three drugs are presented in Fig. 1. The stem of *S. cathayensis* is cylindrically shaped and hairy, 6–9 cm in length and 0.3–1.5 cm in diameter. The branchlets have grey short hairs on their surfaces. The surface is grayish brown with pale yellow lenticels protruding laterally and fine wrinkles longitudinally. The texture of drugs is hard, and they are difficult to break. The decoction pieces are 0.4–1 cm thick, and the cut surface is yellowish. The cortex and pith are brown. The leaves are mostly curled when dry. The shape of leaves has two types. One type is entire, elliptical to oval shaped, 6–12 cm long, and 3.5–7.5 cm wide with an acuminate apex, the base is cuneate or near circular. The other is lobed and present both palmate-unilateral and palmate-bilateral splits, with the two sides of the segments being oblique, the leaf base is wedge-shaped. Leaves have light grey short hairs on both surfaces. The upper leaf surface is grayish green. The lower surface is light green or purple-red. The leaf margin is serrate. The petioles are 2–4 cm long. The leaf is coriaceous. The drugs are slightly fragrant and taste slightly sweet.

Table 1
Collection data for *S. cathayensis*, *P. heterophyllum* and *D. dentiger* samples.

Species	Voucher No.	Medicinal plant parts	Collection date	Source location
Semiliquidambar cathayensis H. T. Chang	S141121	Stems/leaves	November 2014	Ziyuan County, Guangxi Zhuang Autonomous Region
	S150416	Stems/leaves	April 2015	Rongan County, Guangxi Zhuang Autonomous Region
	S141019	Stems/leaves	October 2014	Rongan County, Guangxi Zhuang Autonomous Region
	S141023	Stems/leaves	October 2014	Rongshui County, Guangxi Zhuang Autonomous Region
	S150417	Stems/leaves	April 2015	Ziyuan County, Guangxi Zhuang Autonomous Region
	S141134	Stems/leaves	November 2014	Fangcheng District, Guangxi Zhuang Autonomous Region
Pterospermum heterophyllum Hance	P141122	Stems/leaves	November 2014	Rongan County, Guangxi Zhuang Autonomous Region
	P141125	Stems/leaves	November 2014	Ziyuan County, Guangxi Zhuang Autonomous Region
	P150423	Stems/leaves	April 2015	Rongan County, Guangxi Zhuang Autonomous Region
	P141019	Stems/leaves	October 2014	Rongshui County, Guangxi Zhuang Autonomous Region
Dendropanax dentiger (Harms) Merr.	D141012	Stems/leaves	October 2014	Rongan County, Guangxi Zhuang Autonomous Region
	D141109	Stems/leaves	November 2014	Ziyuan County, Guangxi Zhuang Autonomous Region
	D150419	Stems/leaves	April 2015	Rongshui County, Guangxi Zhuang Autonomous Region
	D150427	Stems/leaves	April 2015	Rongan County, Guangxi Zhuang Autonomous Region

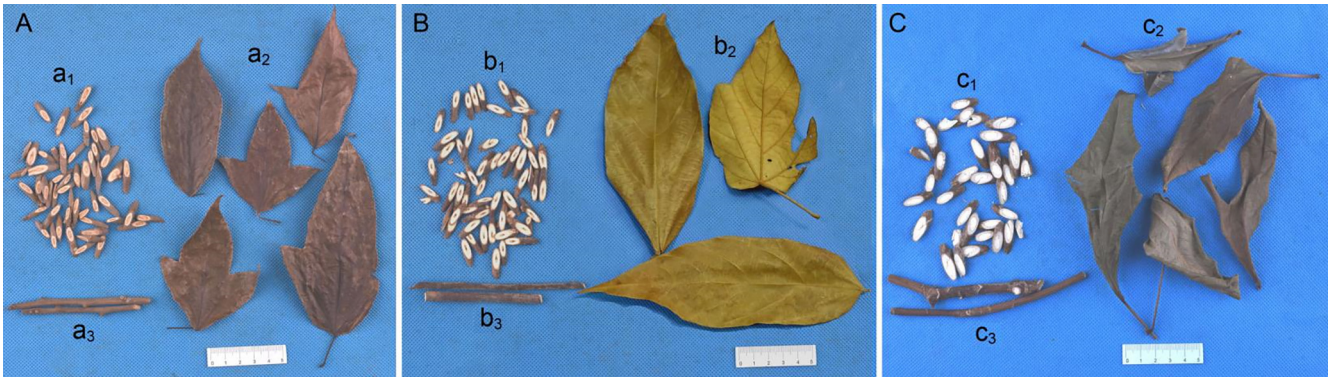

Fig. 1. Decoction pieces of stems (1), leaves (2) and stems (3) of three herbs of *S. cathayensis* (A and a), *P. heterophyllum* (B and b) and *D. dentiger* (C and c).

The stem of *P. heterophyllum* is cylindrically shaped, 6–10 cm long, and 0.3–1.5 cm in diameter. The stem surface is grayish yellow or taupe with fine longitudinal wrinkles. The branchlets have yellow or red hairs on their surfaces. The texture is hard and difficult to break. The decoction pieces are 0.3–0.8 cm thick, and the cut surface is yellowish white. The cortex is brown, and the pith is taupe. The leaves are mostly curled after being dried. The shape of leaves has two types. One type is ovate-lanceolate shaped, 10–17 cm long, and 9–15 cm wide, with three to five lobes in a palmate shape. The bases are truncate or slightly semicircular, and the petioles are 3–12 cm long. The other is round or ovate-oblong, 10–15 cm long, and 5–8 cm wide with a blunt, sharp or acuminate apex, and the base is truncate or obliquely cordate. The petioles are 1–2 cm long. The upper leaf surface is slightly hairy, whereas the lower leaf surface is densely covered with yellowish-brown hairs. The stipules are linear and oblong. The leaf is chartaceous. The smell and taste of drugs are faint.

The stem of *D. dentiger* is cylindrically shaped, and the surface is grayish brown, 8–12 cm in length, and 0.5–2 cm in diameter. Their texture is hard and difficult to break. The pieces of the decoction are 0.5–0.9 cm thick, and the cortex is thin and yellowish-brown. The xylem is small and pale yellow. The pith is large and yellowish white. The leaves curl when dry and vary greatly in shape. The entire leaves are elliptical, ovate-elliptical, ovate-lanceolate, or linear-lanceolate, 5–15 cm long, and 3–10 cm wide. The leaf tip is long and gradually becomes sharper, and the base is obtuse or cuneate. The leaf margin is entire or with several inconspicuous serrations. The lobed leaves are palmately divided with two or three lobes or parted, although a few have five lobes. The leaf margin is entire or with one to several inconspicuous serrations. The leaf bases have red glands and two linear appendages, with triplinerved veins. The upper leaf surface is grayish green, and the lower surface is grayish yellow. Both sides of the leaf are hairless, and the petioles are 2–14 cm long. The leaf is thin and chartaceous. The smell and taste of drugs are faint.

3.2. Microscopic morphological characteristics

3.2.1. Transverse stem sections

The microscopic stem structures of the three medicinal herbs are shown in Fig. 2. *S. cathayensis* has 8–13 rows of flat, square-shaped cork cells. The cork layer is covered with a remnant of single-celled nonglandular hair that is 68–160 μm long. The cortex consists of 10–15 layers of cortical cells that are round or irregularly shaped. The outer cells are arranged closely with thick walls, whereas the inner cells are large and arranged loosely. The pericyclic fibers are intermittently distributed in a circular shape, and the cell wall is thick and lignified. Among them, there are often stone cells, which are square, round, or irregular with thick, lignified walls. The stone cells are different in cavity sizes, with obvious pit canals. The phloem is narrow, and its parenchyma cells are suborbicular in shape. The cambium consists of two rows of cells. The xylem is composed of vessels, wood rays, wood fibers, and wood parenchyma cells. The vessels in the xylem are arranged singly or radially with diameters of 36–75 μm. The wood rays are composed of one to two rows of cells. The pith is broad, and its cells are circular or polygonal. More than 10 schizogenous secretory cavities are arranged alternately in a ring shape of 70–110 μm in diameter. Some parenchyma cells in the cortex and phloem contain calcium oxalate cluster crystals of 15–25 μm in diameter.

P. heterophyllum contains one layer of epidermal cells with linear or stellate nonglandular hairs. The linear nonglandular hairs

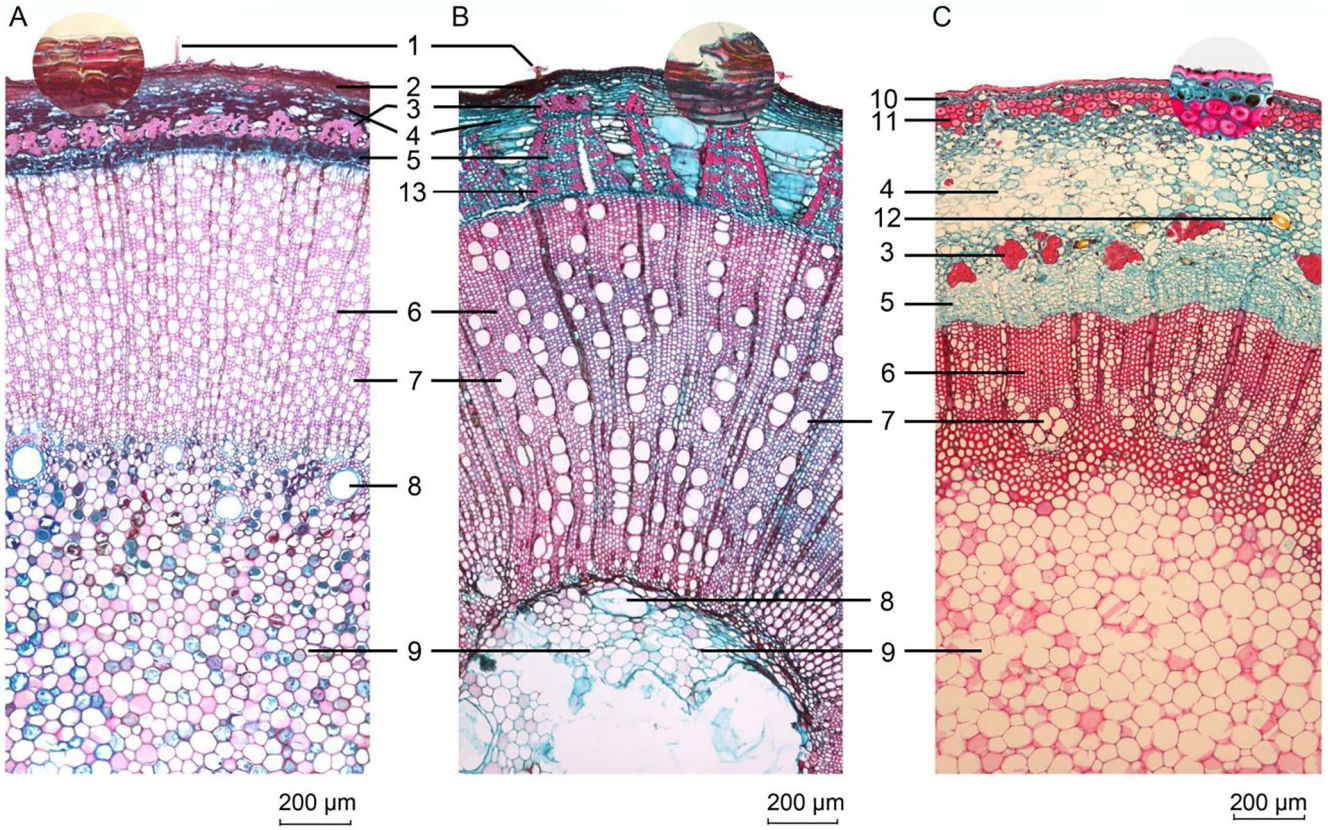

Fig. 2. Microscopic stem structures of three medicinal herbs *S. cathayensis* (A) *P. heterophyllum* (B) and *D. dentige* (C) (×100). 1, Nonglandular hair; 2, phellem; 3, pericyclic fiber; 4, cortex; 5, phloem; 6, xylem; 7, vessel; 8, secretory tissue; 9, pith; 10, epidermis; 11, cortex fiber; 12, resin canal; 13, phloem fiber.

are composed of single cells of 53–150 μm length. The stellate nonglandular hairs are composed of 3–8 body cells and 1–3 stalk cells. The body cells are arranged radially, each cell is lanceolate and some are curved, and the cells vary in length with diameters of 3–18 μm. The cortex cells comprise 7–10 layers, are round or polygonal, and the outer cortex cells are closely arranged with thick walls. Several pericyclic fibers are bundled together and tangentially distributed with thick and lignified walls. The phloem is wide, and the phloem fibers and parenchyma cells are arranged alternately in layers. The phloem fiber wall is thick and lignified, with a small cell cavity. The phloem ray widths vary, some are trumpet-like with scattered schizogenous secretory cavities. The xylem is composed of vessels, wood fibers, wood rays, and wood parenchyma cells, the vessels are arranged singly or with several radial arrangements of 36–115 μm in diameter. The wood rays are composed of one to two rows of cells. The pith is broad, the cells are circular or polygonal and some contain starch. Schizogenous secretory cavities are scattered in the pith, which are 165–215 μm in diameter. Some parenchyma cells in the cortex and phloem contain calcium oxalate cluster crystals of 13–26 μm in diameter.

D. dentiger contains one layer of epidermal cells coated with a thin cuticle outside. The cortex cells are 20–30 layers and are nearly round or square, the outer cortex cells are closely arranged with thick walls. Near the outer side of the cortex, there are one to five layers of fibers intermittently arranged in a ring. The inner cell walls are thin and cells are sparsely arranged. Twenty to thirty resin canals 10–70 μm in diameter are scattered in the cortex. The pericyclic fibers are bundled together, and the cell walls are thick and woody. The phloem parenchyma cells are circular. The xylem is composed of vessels, wood fibers, wood rays, and wood parenchyma cells. The vessels are usually joined and are 36–75 μm in diameter. The wood rays are arranged with one to two rows of cells. The thickness of the lignified walls of the parenchyma cells differs in the xylem. The pith is broad, the cells are circular or polygonal, and the pith cell walls are thick and lignified. Some parenchyma cells in the cortex contain calcium oxalate cluster crystals with diameters of 16–29 μm.

3.2.2. Transverse sections of leaves

The leaf transverse sections of the three medicinal herbs are shown in Fig. 3. *S. cathayensis* comprises two layers of upper epidermal cells, which are rectangular or elliptical and closely arranged. The lower epidermal cells comprise one layer and are small and oblong. Single-celled nonglandular hairs, which are 11–25 μm in diameter and 65–126 μm in length, are found on the epidermis. The leaves are bifacial. The palisade tissue consists of one to three rows of cylindrical cells, which are approximately 50 μm long and do not pass through the midrib. Three to five secretory cavities and canals of different sizes are scattered among parenchymatous cells of the center of vascular cylinder. The vessels in the xylem are radially arranged. The phloem is narrow, and 3–6 rows of pericyclic fibers intermittently surround the bundle. The parenchyma cells contain calcium oxalate cluster crystals of 11–22 μm in diameter.

P. heterophyllum contains one layer of upper and lower epidermal cells in a rectangular shape. Dense single-celled nonglandular hairs or stellate nonglandular hairs occur on the epidermis. Palisade cells are arranged in one to two rows and are cylindrical, approximately 15 μm long, and located away from the midrib. Sponge cells are round with scattered secretory cavities. The vessels are radially arranged with fibers at the center of the xylem.

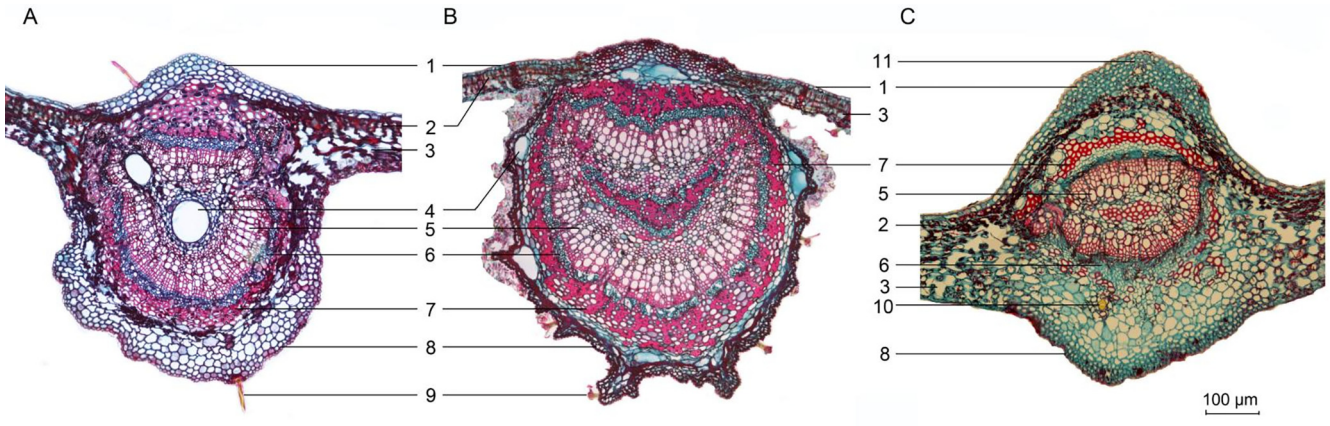

Fig. 3. Leaf transverse sections of three medicinal herbs of *S. cathayensis* (A) *P. heterophyllum* (B) and *D. dentige* (C) (×100). 1, upper epidermis; 2, palisade tissue; 3, spongy tissue; 4, secretory tissue; 5, xylem; 6, phloem; 7, pericyclic fibers; 8, lower epidermis; 9, nonglandular hair; 10, resin canal; 11, collenchyma.

The fiber cells are with lignified walls and arranged in a "V" shape. The phloem is narrow, and 3–10 rows of pericyclic fibers surround the vascular bundle. More than ten secretory cavities of different shapes and sizes surround the vascular cylinder and contain brownish-yellow secretions. The parenchyma cells contain calcium oxalate cluster crystals with diameters of 12–21 μm.

D. dentiger has one layer of upper and lower epidermal cells in a rectangular shape. The palisade tissue cells comprise one to three rows and are short and cylindrical, approximately 30 μm long, and located away from the midrib. There are 4 to 8 rows of collenchyma cells occurring interior to the epidermis of the midrib. The xylem is annular with fibers and parenchyma cells in the middle of vascular cylinder. The vessels are arranged radially. The phloem is narrow, with one to five rows of pericyclic fibers distributed on the outside. Five to eight resin canals are scattered around the vascular cylinder and contain brownish-yellow secretions. Some parenchyma cells contain calcium oxalate cluster crystals that are 12–24 μm in diameter.

3.2.3. Leaf surface slices

The leaf epidermal cells of the three medicinal herbs are shown in Fig. 4. The anticlinal walls of the upper epidermal cells of *S. cathayensis* leaves are curved and thickened. The anticlinal walls of the lower epidermal cells are slightly curved with paracytic stomata that are 13–21 μm in diameter.

The anticlinal walls of the upper epidermal cells of *P. heterophyllum* leaves are relatively straight, and some cells contain brownish-yellow secretions. The anticlinal walls of the lower epidermal cells are slightly curved, with anomocytic stomata of 8–15 μm in diameter. Two types of nonglandular hairs are found on the lower epidermis. One is single-celled nonglandular hair, which is curved, thin, and long. The other is stellate nonglandular hair, which are composed of 3–8 body cells and 1–3 stalk cells, the body cells are arranged radially, each cell is lanceolate and some are curved, and the cells vary in length with diameters of 3–18 μm. The stalk cells are oval.

The anticlinal walls of the upper epidermal cells of *D. dentiger* leaves are slightly curved. The anticlinal walls of the lower epidermal cells are slightly curved with an obvious keratinous texture. The stomata are anomocytic with diameters of 16–24 μm.

3.2.4. Microscopic characteristics of powder

The powder characteristics of the three medicinal herbs are shown in Fig. 5. *S. cathayensis* powder is grayish green. The pericyclic fiber wall is thickened, the cell cavity is narrow, the apex is truncate or round, the length is 580–1360 μm, and the diameter is 18–32 μm. The xylem fiber wall is thin, the cell cavity is large, the length is 260–850 μm, and the diameter is 22–28 μm. The secretory canal is duct-like and contains brownish-yellow materials. Calcium oxalate cluster crystals are scattered, with diameters of 15–39 μm. The stone cells are single, or several are connected. They are square, elliptical, or irregular in shape, with thick walls, obvious pits, and diameters of 25–58 μm. The anticlinal walls of the upper epidermal cells of the leaves are curved and without stomata. The anticlinal walls of the lower epidermal cells of the leaves are slightly curved. Stomata are paracytic, 18–23 μm in diameter. The diameters of spiral vessels are 30–38 μm. The nonglandular hairs are single cells that vary in length, with an acute apex and a diameter of 12–28 μm.

P. heterophyllum powder is yellowish green. The fibers are bundled or separated. The phloem fiber wall is thick, the cell cavity is narrow, and the diameter is 10–22 μm. The xylem fiber wall is thin, the cell cavity is large, and the diameter is 12–25 μm. The secretory cavities are mostly broken, and the intact cavities are rounded with diameters of 165–315 μm and contain brownish-yellow secretions. There are two types of nonglandular hairs, namely, single-celled nonglandular hairs that are curved, thin, and long, and stellate nonglandular hairs that are composed of 3–8 body cells and 1–3 stalk cells. The body cells are arranged radially, each cell is lanceolate and some are curved, and the cells vary in length with diameters of 3–18 μm. The stalk cells are oval. The calcium oxalate cluster crystals are scattered or present in the parenchyma cells with diameters of 13–33 μm. The anticlinal walls of the upper epidermal cells of the leaves are relatively straight, and no stomata are present, whereas the anticlinal walls of the leaf lower epidermal cells are slightly curved. The stomata are anomocytic with diameters of 10–15 μm. The types of vessels are bordered pit or spiral with diameters of 20–31 μm. The simple starch grains are spherical with a diameter of 3–8 μm and a punctate hilum. The compound starch grains are composed of 2–3 granules.

D. dentiger powder is grayish green. The cortex fibers are bundled or separated and broken. The xylem fiber wall is thin, the cell cavity is large, and the diameter is 16–25 μm. The pericyclic fibers are 13–22 μm in diameter with thick wall and a narrow cell cavity. The resin canals are golden yellow or brownish red with diameters of 30–106 μm. The calcium oxalate cluster crystals are scattered or present in parenchyma cells with diameters of 15–28 μm. The anticlinal walls of the upper epidermal cells of the leaves are slightly curved and lack stomata, whereas the anticlinal walls of the lower epidermal cells are slightly curved and covered with an obvious cuticle. The stomata are anomocytic with diameters of

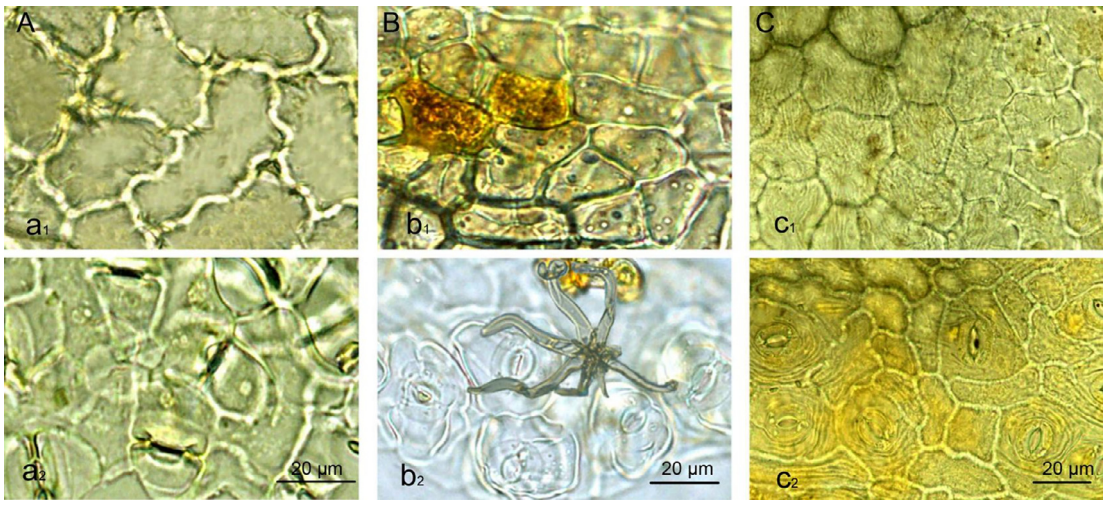

Fig. 4. Surface of leaves of three medicinal herbs of *S. cathayensis* (A) *P. heterophyllum* (B) and *D. dentige* (C) (×400). 1. upper epidermis, 2. lower epidermis.

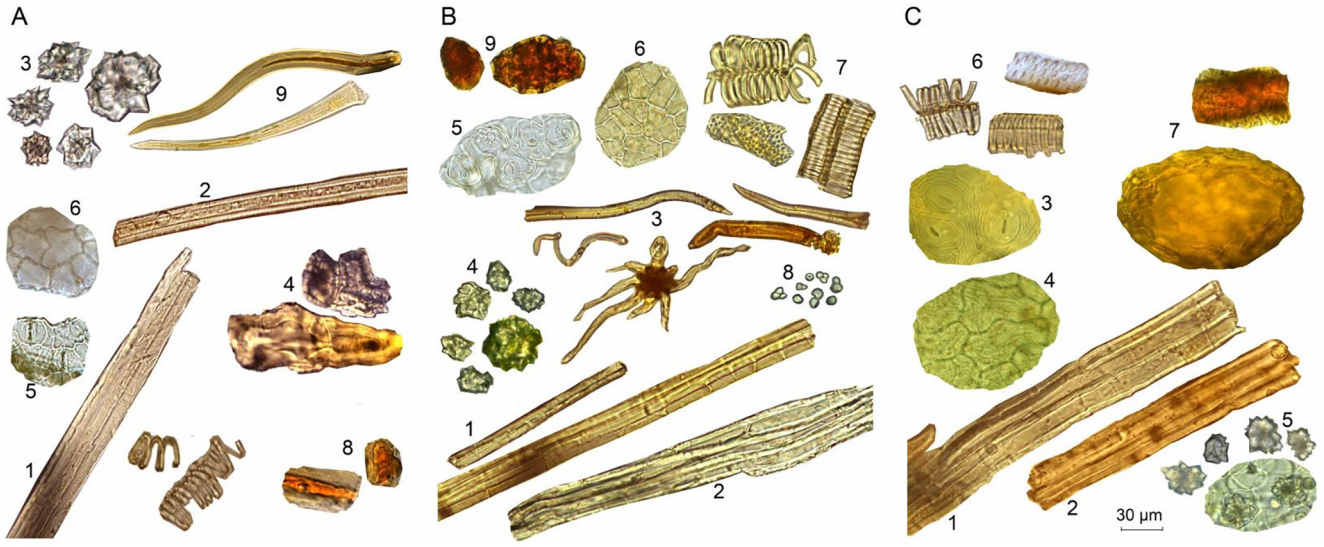

Fig. 5. Microscopical characteristics of powder of three medicinal herbs (×400). A. *S. cathayensis.*; 1, pericyclic fiber; 2, xylem fiber; 3, calcium oxalate cluster crystals; 4, stone cells; 5, leaf lower epidermal cells; 6, leaf upper epidermal cells; 7, vessel; 8, secretory cavity and secretory canal; 9, nonglandular hair. B. *P. heterophyllum*; 1, phloem fiber; 2, xylem fiber; 3, nonglandular hair; 4, calcium oxalate cluster crystals; 5, leaf lower epidermal cells; 6, leaf upper epidermal cells; 7, vessel; 8, starch; 9, secretory cavity fragment. C. *D. dentiger*; 1. xylem fiber; 2, pericyclic fiber; 3, leaf lower epidermal cells; 4, leaf upper epidermal cells; 5, calcium oxalate cluster crystals; 6, vessel; 7, resin canal.

16–23 μm. The types of vessels are bordered pit or spiral with diameters of 35–48 μm.

4. Discussion

Ban Fenghe is an excellent anti-rheumatism Yao medicine in Guangxi Zhuang Autonomous Region, which is derived from the dried stems and leaves of *S. cathayensis.* However, some drugs which are derived from the different species also named "Ban Fenghe", among which *P. heterophyllum* and *D. dentiger* are the most commonly used in the markets of medicinal herbs in Guangxi. The three medicinal herbs have similar morphologies, and it's easy to be confused. To using Ban Fenghe accurately and safely, the macroscopic and microscopic features of these three medicinal herbs from *S. cathayensis*, *P. heterophyllum* and *D. dentiger* were systematically studied in this paper.

Morphologically, the branchlets of *S. cathayensis* have grey short hairs, which are published for the first time. The branchlets of *P. heterophyllum* has yellow or red hairs on their surfaces, while the branchlets of *D. dentiger* have no hair. The stem surface of *S. cathayensis* has transverse lenticels, while this feature is not obvious in the other two drugs. The stem pith of *D. dentiger* is yellow-white, while the other two herbs are nearly brown. The leaves of *S. cathayensis* have light grey short hairs on both surfaces. *P. heterophyllum*'s leaf upper surface is slightly hairy, whereas the lower leaf surface is densely covered with yellowish-brown stellate hairs. Both leaf surfaces of *D. dentiger* are hairless. The leaves of *S. cathayensis* are heterophylly. One type is entire, the other is lobed with both palmate-unilateral and palmate-bilateral splits. The

Table 2
Characteristics for microscopically identifying three medicinal herbs from *S. cathayensis*, *P. heterophyllum* and *D. dentiger*.

Microscopic characteristics	Tissue and cells	*S. cathayensis*	*P. heterophyllum*	*D. dentiger*
Stem transverse sections	Cortex	Narrow	Narrow	Wide, with 1 to 5 layers of fibers intermittently arranged in a ring near outside
	Pericyclic fibers	Intermittently distributed pericyclic fibers in a circular shape, with stone cells	Bundled together, no stone cells	Bundled together, no stone cells
	Phloem	Narrow	Wide, with scattered schizogenous secretory cavities	Wide
	Secretory tissue	Distinct secretory cavities and canals around the pith	Secretory cavities scattered in the pith and phloem	Resin canals distributed in the cortex
Leaf transverse sections	Upper epidermal cells	Two layers	One layer	One layer
	Vascular bundle in midrib	3–5 secretory cavities scattered in the center of vascular cylinder	More than 10 secretory cavities around the vascular cylinder	5–8 resin canals scattered around the vascular cylinder
Powder	Stone cells	Square, elliptical, or irregularly shaped, with thick walls and obvious pit canals	–	–
	Stomata	Paracytic type	Anomocytic type	Anomocytic type
	Nonglandular hair	Single-celled nonglandular hair	Two nonglandular hair types: single-celled and stellate	–
	Secretory tissue	Secretory cavity/Secretory canal	Secretory cavity	Resin canal

Note – means absent.

leaves of *P. heterophyllum* are also heterophylly. One type is ovate-lanceolate shaped, the other is round or ovate-oblong and some of them are lobate. The leaves of *D. dentiger* vary greatly in shape, which includes the entire leaves and the lobed leaves. The entire leaves are elliptical or lanceolate, and the lobed leaves are palmately divided with two to five lobes.

The macroscopic features are often inconspicuous after the drugs being dried and broken, so we carried out microscopic identification study. The microscopic characteristics of these three medicinal herbs from *S. cathayensis*, *P. heterophyllum* and *D. dentiger* are presented in Table 2. The pericyclic fibers of *S. cathayensis* are intermittently distributed in a circular shape and there are some stone cells among them, while pericyclic fibers of *P. heterophyllum* and *D. dentiger* are bundled and there are no stone cells. The secretory tissues in the stems of *S. cathayensis*, *P. heterophyllum* and *D. dentiger* are different. Secretory cavities and canals of *S. cathayensis* are schizogenous and distributed around the pith near the xylem, with diameters of 70–110 μm. *P. heterophyllum* secretory cavities are also schizogenous and are scattered in the phloem and pith, with diameters of 165–215 μm. The resin canals in *D. dentiger* are distributed in the cortex, with diameters of 10–70 μm.

For the features of the leaf transverse sections, three to five secretory cavities and canals are scattered among the center of vascular cylinder of *S. cathayensis*. More than ten secretory cavities of different shapes and sizes surround the midrib of *P. heterophyllum*. The vascular cylinder in the midrib of *D. dentiger* is surrounded by five to eight resin canals. For the features of the leaf surfaces, the anticlinal walls of the upper epidermal cells of *S. cathayensis* leaves are curved, whereas the anticlinal walls of the upper epidermal cells of *P. heterophyllum* and *D. dentiger* leaves are relatively straight. Stomata of *S. cathayensis* are paracytic, whereas stomata of *P. heterophyllum* and *D. dentiger* are anomocytic.

Powder of *S. cathayensis* contains stone cells with thick walls and obvious pit canals, whereas powder of *P. heterophyllum* and *D. dentiger* lack stone cells. *S. cathayensis* powder contains single-celled nonglandular hairs. *P. heterophyllum* powder contains two types of nonglandular hairs: single-celled and stellate. *D. dentiger* powder lack nonglandular hairs. These are important characteristics for distinguishing the three herbs.

5. Conclusion

In summary, we used macroscopic and microscopic identification techniques to study and compare the characteristics of *S. cathayensis*, *P. heterophyllum*, and *D. dentiger*. The morphological identification features can be used to distinguish these three medicinal herbs with the advantages of being fast, simple and reliable. Our study provides a scientific basis for the quality control and clinical use of Ban Fenghe drug from *S. cathayensis*.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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