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Pharmacognostic Study of Five Taxus Seeds

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Abstract:

Objective: To study the seeds and arils of *Taxus wallichiana* var. *chinensis* and other Taxus plants at different levels, to provide a scientific basis for expanding the medicinal resources of *Taxus wallichiana* var. *chinensis*. *Methods:* The five species of Taxus seeds were compared by the traditional methods of character identification and microscopic identification. The main chemical components were detected by UPLC-Q-TOF-MS/MS method, and compared with the reference and known components. *Results:* The five species of Taxus seeds had high similarity in appearance and microscopic characteristics of the seed coat. There were only a few differences, such as seed surface color, seed umbilical shape and microscopic morphology of seed epidermis. After detection and comparison, five samples contained taxane compounds, and there were eleven common components. *Conclusion:* There are few differences in seed morphological characteristics of the five species of Taxus seeds, and they all contain eleven taxanes. The results have guiding significance for the subsequent development and utilization of Taxus medicinal resources.

Keywords:

Taxus
Character identification
Microscopic identification
UPLC-Q-TOF-MS/MS
Taxol

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1. Introduction

Taxus wallichiana var. *chinensis* (Pilg.) Florin is an evergreen tree or shrub belonging to the genus Taxus in the family Taxodiaceae. There are 11 species of this genus worldwide, among which there are 4 species and 1 variant in China, namely, *Taxus chinensis*, *Taxus cuspidata* Siebold & Zucc., *Taxus wallichiana* Zucc., *Taxus yunnanensis* W. C. Cheng & L. K. Fu, and *Taxus wallichiana* var. *mairei* (Lemée & H. Lév.) L. K. Fu & Nan Li [1]. Additionally, Taxus × media Rehder, a natural hybrid species introduced and cultivated in China, has a male parent *Taxus baccata* Thunb. and a female parent *Taxus cuspidata* [2]. Taxus is a globally recognized endangered and rare natural anticancer plant. Paclitaxel, a diterpenoid alkaloid with anticancer activity isolated from the bark of Taxus, has shown significant efficacy in the treatment of various cancers with high incidence rates, such as ovarian cancer, uterine cancer, breast cancer, and non-small cell lung cancer. It is one of the most effective, low-toxic, and broad-spectrum natural anticancer compounds discovered so far [3]. However, due to the extremely low content of natural paclitaxel, which is mostly distributed in the bark of Taxus, the amount of paclitaxel that can be obtained from natural resources is very limited and cannot meet clinical demand. Therefore, researchers have utilized taxane compounds with similar structures to paclitaxel as semi-synthetic raw materials to obtain paclitaxel or more effective anticancer compounds through chemical structural modification [4,5]. Thus, Taxus plants have become medicinal resources with great potential for development and utilization. Besides, the seeds of Taxus plants (including arils) have been used as medicine for a long time. The "Fei Shi" recorded in ancient books comes from the plants of the genus *Taxus* L. [6]. According to the "Dictionary of Chinese Materia Medica," "Xue Fei, the seed of *Taxus wallichiana* var. *mairei*", can "treat food stagnation and expel roundworms" [7]. Since the seeds of Taxus, including their external red arils, are renewable resources that can be collected every year, the rational development and utilization of Taxus plants have become the focus of attention of scholars at home and abroad under the premise of protecting their natural resources. This is also an important reason why Taxus is listed as one of the first provincial precious tree species in Hubei Province [8]. In the view of this, this study intends to conduct systematic pharmacognostic research on seed samples from five Taxus species distributed in the Shennongjia area, analyze and compare the morphological and microscopic characteristics of different samples, and utilize UPLC-Q-TOF-MS/MS technology to analyze and compare the taxane components contained in different samples. The aim is to accumulate the necessary research foundations for the subsequent rational development and utilization of medicinal plant resources of the genus Taxus.

2. Instruments and materials

2.1. Instruments

SZ61 stereomicroscope (Olympus China Co., Ltd.); BM1000 biological microscope (Nanjing Jiangnan Yongxin Optics Co., Ltd.); Agilent 6540 quadrupole-time of flight liquid chromatography-mass spectrometry system, MassHunter chromatography workstation (Agilent Technologies Co., Ltd.); vernier caliper (Guilin Guanglu Digital Measurement and Control Co., Ltd.).

2.2. Reagents

10-deacetylbaccatin III (batch number: H11S9X70-008), 10-deacetylpaclitaxel (batch number: H22M10X88906), 7-epi-10-deacetylpaclitaxel (batch number: R16N11F131203), paclitaxel (batch number: J04S11H123510) were purchased from Shanghai Yuanye Biotechnology Co., Ltd.; baccatin III (batch number: CFS202201), cephalomannine (batch number: CFS202201), 7-xylosyl-10-deacetylpaclitaxel (batch number: CFS202301) were purchased from Wuhan Tianzhi Biotechnology Co., Ltd. Methanol and acetonitrile were of mass spectrometry grade; water was ultrapure water.

2.3. Samples

Some samples were collected from Shennongjia in Hubei and Pan'an in Zhejiang, and some samples were provided by Bozhou Huairentang Chinese Medicinal Materials Company. All samples were identified by Professor Chen Keli from Hubei University of Chinese Medicine, which were seeds and arils of *Taxus wallichiana* var. *chinensis* (Pilg.) Florin, *Taxus cuspidata* Siebold & Zucc., *Taxus wallichiana* Zucc., *Taxus wallichiana* var. *mairei* (Lemée & H. Lév.) L. K. Fu & Nan Li, and *Taxus* × *media* Rehder, respectively.

3. Methods and results

3.1. Morphological identification

The medicinal seeds were all mature products, including intact seeds and arils. In this study, the shape, length, width, color, surface characteristics, and hilum of the seeds were observed and measured, and the morphology of the samples was recorded by photography (**Figure 1**). The similarities and differences in the morphological characteristics of each sample were described and compared.

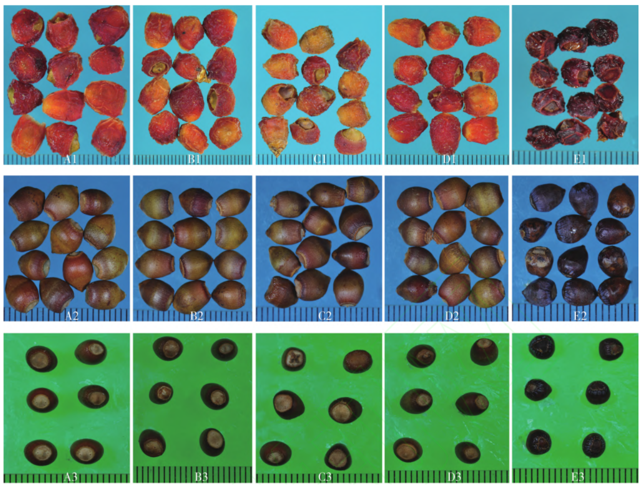

Figure 1. Morphological characteristics of different samples. (A) *Taxus chinensis*; (B) *Taxus cuspidata Siebold & Zucc.*; (C) *Taxus wallichiana Zucc.*; (D) *Taxus wallichiana var. mairei (Lemée & H. Lév.) L. K. Fu & Nan Li*; (E) *Taxus × media Rehder*; (1) Seeds with aril; (2) Seeds; (3) Hilum.

3.1.1. Morphological characteristics of Taxus wallichiana seeds

The overall shape was spherical or elliptical, with a length of about 6.6 to 9.2 mm and a diameter of about 5.5 to 8.2 mm. The outer aril was cup-shaped, red, and fleshy, with a wrinkled surface after drying, a circular opening at the top, and a nearly membranous disc-shaped seed receptacle (i.e., the ovule receptacle that did not develop into a fleshy aril) and fruit stalk at the base. The seeds were mostly oval-shaped, with a pointed upper part and a brownish-yellow surface; they were about 6.0 to 7.7 mm long and 4.6 to 5.7 mm wide. The cross-section was oblate elliptical, often with two obtuse ridges on the upper part and a protruding short tip at the top. The hilum was mostly wide elliptical, rarely triangular-round, with a protruding center.

3.1.2. Comparison of morphological characteristics of different samples

The appearance, morphological characteristics, and sizes of different samples were similar, but there were still differences in some details. To understand the differences in morphological characteristics of different samples more clearly, a comparison was conducted in this study (**Table 1**). The results showed that the seeds of Taxus × media were the smallest, and the surface of the aril was dark purplish red. The surface of the aril of *Taxus wallichiana* Zucc. was light reddish-yellow, while the arils of the

Table 1. Comparison of trait characteristics of different samples

Category	Taxus chinensis	*Taxus cuspidata* Siebold & Zucc.	*Taxus wallichiana* Zucc.	*Taxus wallichiana var. mairei* (Lemée & H. Lév.) L. K. Fu & Nan Li	*Taxus × media* Rehder
Seed length	6.6–9.2 mm	6.6–9.6 mm	6.5–8.3 mm	6.2–9.3 mm	5.3–7.2 mm
Seed width	5.5–8.2 mm	5.5–9.1 mm	5.5–7.3 mm	5.5–7.8 mm	5.1–6.4 mm
Seed color	The surface is tan	The surface is tan	The surface is tan	The surface is tan	The surface is dark purple
Seed shape	Oval-shaped, with a gradually tapering top, a short point at the apex, and often with 2 obtuse edges on the upper part	Oval-shaped, with a short point at the apex and 2–4 obtuse edges on the upper part	Oval or broadly oval-shaped, with a short point at the apex and 2–4 obtuse edges on the upper part	Oval or slightly obovate, with a short point at the apex and often with 2 obtuse edges on the upper part	Broadly oval or ovate-triangular shaped, with a short point at the apex and 2–4 obtuse edges on the upper part
Seed cross-section	Flat oval shape	Oval or almost round shape, often with a purple ring at the base	Broadly oval or almost round shape	Oval or almost round shape, with a purple ring partially visible at the base	Almost round shape
Shape of hilum	Mostly wide oval, or triangular round with a convex center	Almost round, square, or oval	Almost round, square, or broadly oval	Oval	Round or triangular round

other three plant seeds were bright red. The upper parts of the seeds of *Taxus wallichiana* var. *chinensis* and *Taxus wallichiana* var. *mairei* had two obtuse ridges, while the other three species showed 2 to 4 ridges. The bases of the seeds of *Taxus wallichiana* var. *mairei* and *Taxus cuspidata* had distinct purple rings, while the other three species did not show obvious purple rings.

3.2. Microscopic identification

The seeds and arils of different samples were made into paraffin sections by referencing the methods of Chinese medicine identification, and the tissue characteristics of each part were observed under a microscope and photographed to record the data.

3.2.1. Tissue characteristics of the cross-section of the aril and seed of Taxus wallichiana

The tissue characteristics of the cross-section of *Taxus wallichiana* and other samples are shown in **Figure 2**. The tissue characteristics of the cross-section of the aril and seed of *Taxus wallichiana* are described below: The outer epidermal cells of the aril were arranged in a single row, with an unevenly thickened cuticle on the surface, forming papillae-like protrusions (**Figure 2A$_1$**). The inner epidermal cells of the aril were also arranged in a single row, appearing round-like (**Figure 2A$_2$**). The intermediate layer between the inner and outer epidermis of the aril was composed of parenchyma cells, with visible vessels inside. The outermost layer of the seed coat consisted of a single row of nearly square, three-sided thickened stone cells, which were slightly radially extended. The outer and lateral walls were thickened, with a length of about 91 μm, a width of about 66 μm, and a wall thickness of about 20 μm. Beneath the stone cells, there was a single row of flat, brown, thick-walled pigment cell layers with inconspicuous cell cavities. Inside the pigment layer was a radially extended, thick-walled, and narrow-cavity palisade stone cell layer. The inner stone cells were arranged in multiple rows, polygonal-like, with thick walls and obvious, round-hole-shaped cell cavities. The outer side of the endosperm consisted of a single row of flat parenchyma cells, appearing irregularly oblong or polygonal (**Figure 2A$_3$**). The endosperm cells were round or polygonal-like, relatively large, and contained aleurone grains (**Figure 2A$_4$**). The center was the embryo, with smaller cells appearing elliptical or round-like (**Figure 2A$_5$**).

3.2.2. Comparison of the aril and seed cross-sections of different samples

To gain a clearer understanding of the similarities and differences in the tissue characteristics of the cross-sections of different samples, this study conducted a detailed comparison of five species (**Table 2**). The results indicate a high degree of consistency in the tissue structure features of the aril, seed coat, endosperm,

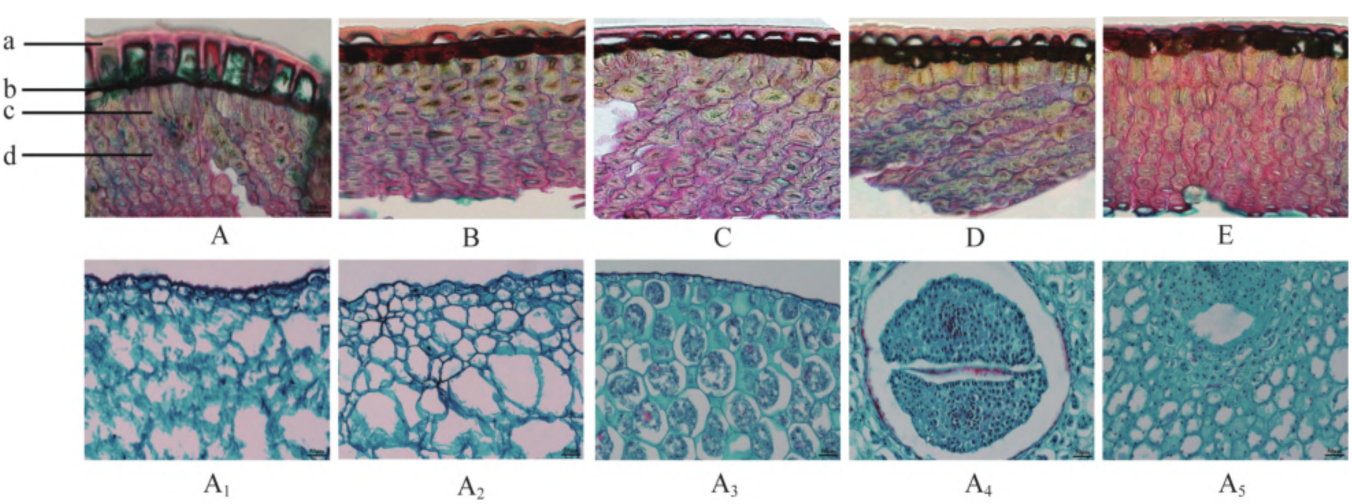

Figure 2. Microscopic characteristics of the cross-sectional tissue of different samples. (A) *Taxus chinensis* (a: stone cells in the outer layer of the seed coat; b: pigment cell layer; c: palisade cell layer; d: inner stone cells); (A$_1$) Outer epidermis of the aril; (A$_2$) Inner epidermis of the aril; (A$_3$) Outer endosperm; (A$_4$, A$_5$) Embryo; (B) *Taxus cuspidata* Siebold & Zucc.; (C) *Taxus wallichiana* Zucc.; (D) *Taxus wallichiana var. mairei* (Lemée & H. Lév.) L. K. Fu & Nan Li; (E) *Taxus* × *media* Rehder.

and embryo among the five samples, with very similar morphologies observed in the outer stone cells and inner stone cells of the seed coat. However, there are a few differences. For instance, in the *Taxus chinensis*, the pigment cell layer below the thickened stone cell layer on the outer side of the seed coat is very thin, and the cell cavity is not prominent. In contrast, the pigment cell layer is thicker and the cell cavity is more evident in the other four congeneric species. Additionally, the outer stone cells in the cross-section of the *Taxus chinensis* and *Taxus wallichiana var. mairei* (Lemée & H. Lév.) L. K. Fu & Nan Li seeds appear radially extended, while those of the other three plant seeds are tangentially extended. Moreover, the palisade stone cells in the seeds of *Taxus cuspidata* Siebold & Zucc. and *Taxus wallichiana* Zucc. do not form a separate column but are arranged intermittently, whereas those in the other three species form a distinct column with narrow cells and cavities arranged tightly.

3.3. Analysis of chemical components in Taxus chinensis seeds

3.3.1. Preparation of test solution

Powdered samples of *Taxus chinensis* seeds (including the aril) from different production areas were precisely weighed, with 1.0 g of each sample placed in a 50 mL centrifuge tube. 25 mL of 70% ethanol was added, and the mixture was extracted for 2 hours using ultrasound (250 W, 40 kHz). The supernatant was collected after filtration and the extraction process was repeated three times. The supernatants were combined, evaporated to dryness in a water bath, dissolved in methanol, and diluted to 10 mL. The solution was filtered through a 0.22 μm microporous membrane, and the filtrate was set aside for use.

Table 2. Comparison of cross-sectional tissue characteristics of different samples

Category	Taxus chinensis	*Taxus cuspidata* Siebold & Zucc.	*Taxus wallichiana* Zucc.	*Taxus wallichiana var. mairei* (Lemée & H. Lév.) L. K. Fu & Nan Li	*Taxus × media* Rehder
Inner sclerenchyma cells	The inner sclerenchyma cells are arranged in multiple rows, polygon-like, with thickened walls and distinct, round pores in the cell cavity.				
Endosperm	The outer layer of the endosperm consists of a single row of flat, thin-walled cells, which are irregularly oblong or long polygon-shaped. The endosperm cells are round or polygon-like, relatively large, and contain aleurone grains.				
Embryo	The center contains the embryo, with smaller cells that are elliptical or round-like.				
Outer sclerenchyma cell layer of seed coat	The outer side comprises a single row of radially extended sclerenchyma cells with thickening on three sides.	The outer side comprises a single row of sclerenchyma cells with thickening on three sides, slightly radially extended.	The outer side comprises a single row of sclerenchyma cells with thickening on three sides, tangentially extended.	The outer side comprises a single row of sclerenchyma cells with thickening on three sides, tangentially extended.	The outer side comprises a single row of sclerenchyma cells with thickening on three sides, tangentially extended.
Pigment cell layer	A single row of brown pigment cells, with a relatively thin pigment cell layer and indistinct cell cavities.	A single row of brown pigment cells, with a thicker pigment cell layer and distinct cell cavities	A single row of brown pigment cells, with a thicker pigment cell layer and distinct cell cavities	A single row of brown pigment cells, with a thicker pigment cell layer and distinct cell cavities	A single row of brown pigment cells, with a thicker pigment cell layer and distinct cell cavities
Palisade sclerenchyma cell layer	A single row of palisade sclerenchyma cells with narrow cell cavities.	A single row of palisade sclerenchyma cells with narrow cell cavities.	Scattered palisade sclerenchyma cells with intervals, and narrow cell cavities.	Scattered palisade sclerenchyma cells with intervals, and narrow cell cavities.	A single row of palisade sclerenchyma cells with narrow cell cavities.

3.3.2. Preparation of reference solution

Appropriate amounts of paclitaxel, 10-deacetylbaccatin III, cephalomannine, baccatin III, 7-xylosyl-10-deacetylpaclitaxel, 10-deacetylpaclitaxel, and 7-epi-10-deacetylpaclitaxel were precisely weighed and dissolved in methanol to prepare a mixed reference solution with a concentration of approximately 50 μg/mL.

3.3.3. Chromatographic and mass spectrometric conditions

An Agilent ZORBAX RRHD SB-C18 (100 mm × 2.1 mm, 1.8 μm) chromatographic column was used. The mobile phase consisted of (A) acetonitrile and (B) 0.1% formic acid in water, with a gradient elution program (0–2 min, 5–10% A; 2–4 min, 10–25% A; 4–35 min, 25–95% A; 35–38 min, 95–95% A; 38–39 min, 95–5% A; 39–40 min, 5–5% A). The flow rate was set at 0.2 mL/min, the column temperature was maintained at 40°C, and the injection volume was 2 μL. A heated electrospray ionization source (H-ESI) was employed in both positive and negative ion scanning modes. The sheath gas temperature was set at 280°C, the sheath gas flow rate was 11 L/min, the nozzle voltage was 1000 V (negative ion mode: -1500 V), the nebulizer gas pressure was 40 psi, and the capillary voltage was 4000 V (negative ion mode: -3500 V). The scanning range was from m/z 50 to 1500.

3.3.4. Data analysis

A first-order mass spectrometry database for Taxus plants was constructed based on literature, including information such as names, chemical formulas, relative molecular masses, and structural formulas. The MassHunter Qualitative Analysis B.08.00 software was used to process the chromatographic peaks detected in the total ion chromatograms of the extract solutions from the five different Taxus species. In the first-order mass spectra, the molecular formulas were determined based on the exact mass numbers, isotope abundance ratios, and error ranges ($\leq 5 \times 10^{-6}$) of the chromatographic peaks. In the second-order mass spectra, fragment information for specific molecular ions within a given retention time range was obtained. Chemical structures for each peak were inferred based on various databases, and chemical components were analyzed and identified using reference standards, PubChem, MassBank of North America, and other databases.

3.3.5. UPLC-Q-TOF-MS/MS results

The test solutions were analyzed according to the chromatographic and mass spectrometric parameters described in section 3.3.3., resulting in BPI charts for both positive and negative ion modes (**Figure 3**). 11 taxane compounds were initially identified and analyzed (**Table 3**).

Table 3. Information on taxanes commonly found in different samples

Serial number	Retention time/ min	Molecular formula	Ion mode	Compound name	Compound type	Basis for comparison
1	9.71	$C_{29} H_{36} O_{10}$	$[M+H]^+$	10-deacetylbaccatin III	Taxanes	Reference
2	12.04	$C_{37} H_5 NO_9$	$[M+H]^+$	Taxuspine Z	Taxanes	[9]
3	12.95	$C_3 H_{38} O_{11}$	$[M+H]^+$	Baccatin III	Taxanes	Reference
4	15.07	$C_{50}H_{57}NO_{17}$	$[M+H]^-$	7-xylosyl-10-deacetyltaxol	Taxanes	Reference
5	15.50	$C_{28}H_{38} O_{10}$	$[M+H]^+$	Taxuspine F	Taxanes	[9]
6	16.76	$C_4 H_{49}NO_{13}$	$[M+H]^-$	10-deacetyltaxol	Taxanes	Reference
7	18.62	$C_{45}H_{53} NO_{14}$	$[M+H]^+$	Cephalomannine	Taxanes	Reference
8	18.95	$C_{26}H_{36} O_8$	$[M+H]^+$	Taxinine A	Taxanes	[9]
9	19.25	$C_4 H_{49} NO_{13}$	$[M+H]^-$	7-epi-10-deacetyltaxol	Taxanes	Reference
10	19.26	$C_{47}H_{51} NO_{14}$	$[M+H]^+$	Paclitaxel	Taxanes	Reference
11	23.38	$C_{37} H_{44} O_{11}$	$[M+H]^+$	Taxinine B	Taxanes	[10]

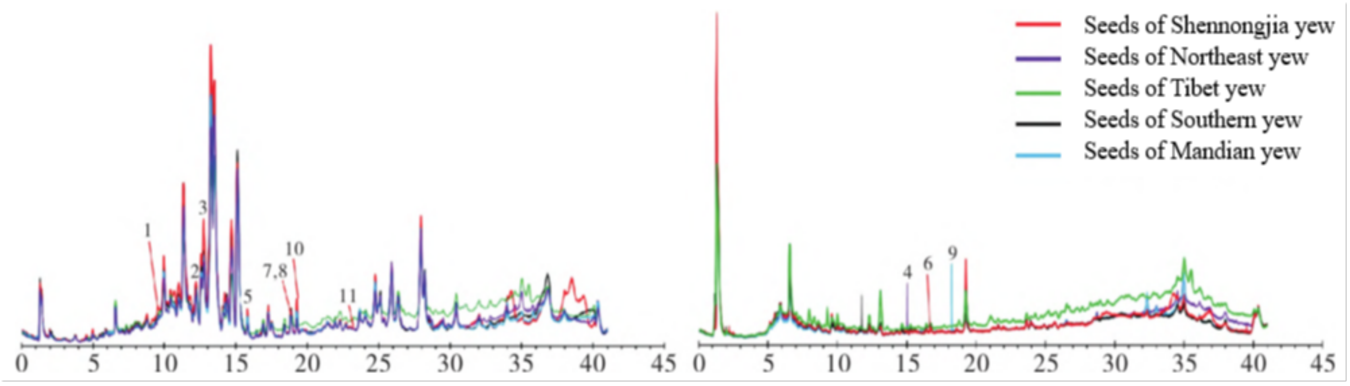

Figure 3. Total ion chromatograms of different samples in positive ion mode (ESI+) and negative ion mode (ESI-).

4. Discussion

During a field study in 1994, the Hubei Provincial Forestry Academy discovered that *Taxus chinensis* is distributed in Shennongjia and its adjacent Western Hubei region. However, due to the relatively slow growth rate of the *Taxus chinensis* and the severe damage to wild *Taxus chinensis* resources in recent years, finding and expanding new sources of *Taxus chinensis* has become an important way to address the supply and demand contradiction while protecting and restoring wild *Taxus chinensis* resources. In this study, seed traits, microscopic identification, and taxane chemical components were identified for five different Taxus species. The results showed a high degree of similarity in the morphological characteristics and microscopic tissue structure of the seeds' cross-sections among these five Taxus species. Although there are some differences between species, the results obtained from UPLC-Q-TOF-MS/MS detection indicate that all five Taxus species contain 11 taxane compounds in their seeds. As the most important active ingredient, taxanes have strong antitumor activity, with paclitaxel being the most significant. It has been widely used internationally for the treatment of various tumors such as ovarian cancer, cervical cancer, endometrial cancer, breast cancer, lung cancer, melanoma, and pancreatic cancer [11]. Additionally, taxane compounds such as 10-deacetylbaccatin III, baccatin III, and cephalomannine are precursors for the synthesis of paclitaxel. Therefore, the results of this study provide data support for further development and utilization of closely related plant resources of the *Taxus chinensis* and experimental evidence for further searching for natural active ingredients. This, to some extent, will help alleviate the shortage of paclitaxel resources and improve the utilization rate of existing *Taxus chinensis* resources. In the later stage, the research group will further analyze the main active ingredients and study their pharmacological activities based on this, providing more scientific evidence for the sustainable development and utilization of medicinal resources of the Taxus genus.

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Disclosure statement

The authors declare no conflict of interest.

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