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Pharmacognostic Study of Sterculia Seed Post-print

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Abstract

Sterculia nobilis possesses stomach-warming and antiparasitic efficacies; however, its name and macroscopic characteristics are easily confused with other species in the Sterculia genus, and the relevant research foundation remains relatively weak. Pharmacognostical studies can provide valuable references for its resource development and quality standard establishment. This study investigated the macroscopic characteristics, micro-morphological characteristics, seed transverse sections, and powder microscopic features of Sterculia nobilis; DNA barcode sequences (ITS2, psbA-trnH, matK, and rbcL) were obtained via bidirectional sequencing, Kimura 2-Parameter genetic distances were calculated, and a neighbor-joining phylogenetic tree was constructed for cluster analysis. The results demonstrated: (1) Macroscopic characteristic features of Sterculia nobilis include external deep-red pericarp coverage, a seed surface that is reddish-brown or dark chestnut-colored, hard texture, and the presence of two pieces of light-yellow thick endosperm. (2) Micro-morphological characteristic features comprise a reddish-brown testa that is extremely thin and brittle; a dark brown middle seed coat that is relatively thick and hard; and a light-yellow inner seed coat that is soft. (3) Microscopic features include the structure and arrangement of stone cells in the testa, palisade cell structure in the middle seed coat, beaded thickening of cell walls in the inner seed coat, and calcium oxalate cluster crystals. (4) Based on the ITS2 sequence, Sterculia nobilis can be effectively distinguished from other Sterculia species, while the matK sequence can effectively differentiate Sterculia lanceolata from other Sterculia species. The obtained macroscopic, micro-morphological, and microscopic characteristic data for Sterculia nobilis, combined with the ITS2 barcode sequence, can effectively authenticate this species, providing a scientific basis for its resource development and quality standard establishment.

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Preamble

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Pharmacognostical Study of Sterculia nobilis Fruit

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Abstract

Sterculia nobilis fruit possesses therapeutic effects such as warming the stomach and eliminating parasites. However, its name and morphological characteristics are easily confused with other *Sterculia* species, and relevant research remains limited. Pharmacognostical studies can provide valuable references for resource development and quality standard formulation. This study systematically investigated the macroscopic, micro-morphological, and microscopic characteristics of Sterculia nobilis fruit, including seed transverse sections and powder features. DNA barcode sequences (ITS2, psbA-trnH, matK, and rbcL) were obtained through bidirectional sequencing, Kimura 2-Parameter genetic distances were calculated, and neighbor-joining phylogenetic trees were constructed for cluster analysis. The results demonstrated: (1) Macroscopic characteristics include a dark red pericarp, reddish-brown or dark chestnut seed surface, hard texture, and two thick, light-yellow endosperm lobes. (2) Micro-morphological features consist of a reddish-brown, extremely thin and brittle exotesta; a black-brown, thick and hard mesotesta; and a soft, light-yellow endotesta. (3) Microscopic identification markers include the structure and arrangement of exotesta stone cells, mesotesta palisade cells, beaded thickening of endotesta cell walls, and calcium oxalate cluster crystals. (4) The ITS2 sequence effectively distinguished Sterculia nobilis from other *Sterculia* species, while the matK sequence effectively differentiated *Sterculia lanceolata* from other *Sterculia* species. The integrated data on morphological, micro-morphological, and microscopic characteristics, combined with ITS2 barcode sequences, provide a scientific basis for the authentication of Sterculia nobilis and the establishment of quality standards.

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Keywords: Sterculia nobilis fruit; character identification; micro-morphological identification; microscopic identification; DNA barcoding

Introduction

Sterculia nobilis fruit is the dried mature seed of *Sterculia nobilis* Smith, belonging to the family Sterculiaceae. Commonly known as "Jiucengpi," "Pingpoguo," "Pinpoguo," "Luowangzi," "Luohuangzi," "Qijioguo," and "Fuguizi" (Jiangsu New Medical College, 1985), it was first recorded in the Song Dynasty text *Guihai Yuheng Zhi* by Fan Chengda (1986), which noted: "Luohuangzi resembles an olive, with seven layers of skin." *Chinese Materia Medica* Volume 5 (1998) lists Sterculia nobilis fruit as a medicinal herb with stomach-warming and antiparasitic effects, used to treat vomiting due to stomach cold, abdominal pain from parasitic accumulation, hernia pain, and infantile scalp ulcers. Modern research indicates that Sterculia nobilis fruit contains abundant polyphenols with strong antioxidant activity (Zhang et al., 2018), demonstrating antioxidant pharmacological effects. The fruit is relatively large, with diameters reaching 2.0–2.5 cm, containing 1–5 seeds per follicle, with an edible portion comprising 82% of the seed and rich nutritional value (Wang, 2002).

Sterculia nobilis is a widely distributed arbor in the Lingnan region with a long cultivation history. It is primarily cultivated in Guangdong, southern Guangxi, southeastern Fujian, southern Yunnan, and Taiwan, particularly in Guangzhou and the Pearl River Delta region. It is also distributed in India, Vietnam, and Indonesia, mostly through artificial cultivation (Flora of China Commission, 1984). The source plant is easily confused with congeneric species such as *S. lanceolata*, *S. lanceaefolia*, and *S. hymenocalyx*, which have similar names. Among these, *S. lanceolata* is the most widely distributed and abundant species in the genus (Su et al., 2019), but its seeds are not used medicinally. Therefore, a more objective and accurate identification method is needed to supplement traditional approaches.

DNA barcoding molecular identification utilizes a standardized, relatively short DNA sequence from the genome for species identification, serving as an effective complement to traditional morphological methods (Chen et al., 2013). For plant-derived Chinese medicinal materials, ITS2 is recommended as the primary barcode sequence with psbA-trnH as a supplementary sequence (Chen et al., 2010), meeting the requirements of simplicity and precision in identification with clear criteria for accurate authentication of medicinal materials and their source species.

Currently, no national standards or literature describe the pharmacognostical characteristics of Sterculia nobilis fruit, and no relevant quality standards exist. Furthermore, the phylogenetic relationships between Sterculia nobilis fruit and its closely related *Sterculia* species remain unclear, with no reports on suitable DNA barcodes for its molecular identification, hindering practical authentication work and germplasm resource development.

Therefore, this study systematically investigated the macroscopic, micromorphological, and histological microscopic characteristics of Sterculia nobilis fruit using normal and polarized light microscopy to supplement and 完善 the holographic color image database for macroscopic and microscopic identification features. Additionally, cluster analysis was performed on seven *Sterculia* species from the Sterculiaceae family based on four universal DNA barcode sequences (ITS2, psbA-trnH, matK, and rbcL) to explore the phylogenetic relationships between Sterculia nobilis fruit and other *Sterculia* species and to screen for suitable DNA barcodes for authenticating the source species. This research provides a scientific foundation for establishing and improving quality standards for Sterculia nobilis fruit and promoting resource development.

Materials and Methods

1.1.1 Medicinal Materials and Sequences

Samples were collected from Guangdong and Guangxi provinces (Table 1) and identified by Professor Wu Wenru from the Department of Authentication of Chinese Medicines at Guangzhou University of Chinese Medicine as dried mature seeds and fresh leaves of *Sterculia nobilis* Smith and fresh leaves of *Sterculia lanceolata* Cav. Samples were stored at the Laboratory of Authenticity Identification and Quality Evaluation of Chinese Medicinal Materials, Guangzhou University of Chinese Medicine, at 4°C.

A total of 92 valid barcode sequences for *Sterculia* species and adulterants, including *S. nobilis*, *S. lanceolata*, *S. brevissima*, *S. lanceaefolia*, *S. africana*, *S. pexa*, and *S. hymenocalyx*, were downloaded from the GenBank database (Table 2).

1.1.2 Reagents

Chloral hydrate (analytical grade, Shanghai Macklin Biochemical Technology Co., Ltd.); dilute glycerin (analytical grade, Tianjin Zhiyuan Chemical Reagent Co., Ltd.); plant genomic DNA extraction kit (spin column type) [Tiangen Biotech (Beijing) Co., Ltd.]; 6× DNA loading buffer (Beijing Solarbio Science & Technology Co., Ltd.); DNA molecular weight marker (100–2000 bp) [Sangon Biotech (Shanghai) Co., Ltd.]; agarose (Beijing Lanjieke Technology Co., Ltd.); 50× TAE electrophoresis buffer (Beijing Zhuangmeng International Biotechnology Co., Ltd.); DNA Gel Stain (GlpBio, USA); Ex Taq (Premix) PCR system [Takara Bio (Beijing) Co., Ltd.]; primers synthesized by Beijing Liuhe Huada Gene Technology Co., Ltd.

1.1.3 Instruments

Canon S120 digital camera (Canon, Japan); CryoStar NX50 cryostat (Thermo Scientific, USA); SZ680 continuous zoom stereomicroscope (Chongqing Optec Instrument Co., Ltd.); OPTEC BK5000 biological microscope (Chongqing Optec Instrument Co., Ltd.); OPTPro3 digital imaging system (Chongqing Optec Instrument Co., Ltd.); XS125A electronic analytical balance [Precisa International Trading (Shanghai) Co., Ltd.]; HH-24 digital constant temperature water bath [Oulade Scientific Instrument (Beijing) Co., Ltd.]; 1-14 benchtop high-speed centrifuge (Sigma Zentrifugen, Germany); RePure-B gradient PCR instrument (Shanghai Chuangmeng Biotechnology Co., Ltd.); JS-2012 gel imaging system (Shanghai Peiqing Technology Co., Ltd.); Power Pac Basic horizontal electrophoresis unit (Bio-Rad, USA); D1008E palm centrifuge (Scilogex, USA); VORTEX 1 vortex mixer [IKA (Guangzhou) Instrument Equipment Co., Ltd.].

1.2.1 Macroscopic Identification

Sterculia nobilis fruit samples were systematically observed for shape, size, color of different parts, surface characteristics, texture, and odor of both seed coat and endosperm. High-resolution digital photography was used to record images, which were subsequently processed using Adobe Photoshop 2022 for enlargement, background removal, and stitching to obtain holographic color image data.

1.2.2 Micro-morphological Identification

Samples were examined under a stereomicroscope with adjusted illumination and focus to observe and record the seed coat structure and micro-morphological characteristics of each layer, generating holographic color image data.

1.2.3 Microscopic Identification

1.2.3.1 Seed Transverse Section Microscopic Identification

Seed transverse sections were prepared using a cryostat, cleared with chloral hydrate, and observed under a biological microscope to obtain holographic color image data.

1.2.3.2 Powder Microscopic Identification

Appropriate amounts of sample were ground, passed through a 60-mesh sieve, cleared with chloral hydrate, and observed under both normal bright field and polarized dark field microscopy. Large-image stitching combined with real-time depth-of-field extension imaging technology was employed to capture holographic color image data.

1.2.4 DNA Extraction, PCR Amplification, and Sequencing

Approximately 100 mg of each of the four samples listed in Table 1 was ground in liquid nitrogen in a mortar, transferred to 1.5 mL centrifuge tubes, and total DNA was extracted using a spin column plant genomic DNA extraction kit. DNA quality was verified by 1% agarose gel electrophoresis. Primers and PCR conditions for ITS2, psbA-trnH, matK, and rbcL sequences are listed in Table 3 . The 50 L PCR reaction mixture contained: Ex Taq (Premix) 25 L, forward and reverse primers 1 L each, DNA template 2 L, and ddH_2O 21 L. Amplification conditions: initial denaturation at 95°C for 4 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing for 1 min (temperatures listed in Table 3), extension at 72°C for 1 min, and final extension at 72°C for 10 min. ddH_2O served as the negative control. Amplified products were visualized and photographed using a gel imaging system. PCR products with single, clear, and bright bands were sent to Guangzhou Sequencing Department of Beijing Liuhe Huada Gene Technology Co., Ltd. for bidirectional sequencing.

1.2.5 Sequence Analysis and Species Identification

Sequencing chromatograms were assembled and proofread using SeqMan software to remove primer and low-quality regions and obtain complete sequences. Combined with sequences obtained from GenBank, multiple sequence alignment was performed using ClustalW in MEGA 11 software to analyze intraspecific and interspecific genetic variation. Kimura 2-Parameter (K2P) genetic distances were calculated, neighbor-joining (NJ) phylogenetic trees were constructed, and bootstrap support values (1,000 replicates) were used to test branch reliability.

Results

2.1 Macroscopic Identification

Sterculia nobilis fruit is covered by a dark red pericarp (Figure 1 [Figure 1: see original paper]: B). Seeds are elliptical or spindle-shaped, 2–3 cm in length and 1–2 cm in diameter, with a blunt apex and slightly pointed base (Figure 1: E). The hilum is yellowish-white and circular (Figure 1: C). The seed surface is reddish-brown or dark chestnut, slightly lustrous, with irregular shrinkage wrinkles and a hard texture (Figure 1: D). Transverse section reveals thick, light-yellow endosperm consisting of two lobes, broadly ovate, with a mucilaginous surface (Figure 1: F). The fruit has a faint odor and mild taste.

2.2 Micro-morphological Identification

The seed coat of Sterculia nobilis consists of three layers: exotesta, mesotesta, and endotesta (Figure 2 [Figure 2: see original paper]). The exotesta is reddish-brown, extremely thin, brittle, easily detached, with irregular shrinkage wrinkles and slight luster (Figure 3 [Figure 3: see original paper]: A, B). The mesotesta is black-brown, relatively thick, hard, with fine irregular wrinkles and no luster

(Figure 3: C, D). The endotesta is light yellow, soft, easily separated from the mesotesta, and covered with a transparent film (Figure 3: E, F).

2.3.1 Seed Transverse Section Microscopic Identification

The exotesta consists of a single layer of square-shaped stone cells arranged tightly with extremely thick outer walls; the cell cavity is 偏向内侧 (shifted inward) and contains brown substances, with a diameter of 20–30 m. The hypodermal layer comprises 2–3 rows of irregularly arranged yellowish-brown cells (Figure 4 [Figure 4: see original paper]: C). Several rows of thin-walled exotesta cells are light yellow, round, square, or irregularly polygonal, with slightly thickened walls, large cavities, some containing brown substances, measuring 50–60 m tangentially and 30–40 m radially (Figure 4: D). The mesotesta palisade cell layer consists of two interlocking rows of light yellow cells containing brown substances, measuring 280–300 m radially (Figure 4: E). The pigment layer comprises several rows of irregularly shaped, yellowish-brown cells with extremely thick walls, arranged irregularly (Figure 4: F). Endotesta cells are square or polygonal with beaded wall thickening, measuring 10–15 m radially (Figure 4: G). Several rows of thin-walled endotesta cells are light yellow, square, irregularly polygonal, or round, with large cavities, occasionally containing brown substances, measuring 60–80 m tangentially and 40–50 m radially (Figure 4: H).

2.3.2 Powder Microscopic Identification

Sterculia nobilis fruit powder is brownish. Under normal light, mesotesta palisade cells appear light yellow in surface view, with thickened walls, polygonal shape, and small circular or flattened cavities (Figure 5 [Figure 5: see original paper]: A). In side view, they appear light yellow, elongated columnar, palisade-like, 280–300 m in length, with cavities containing yellowish-brown substances (Figure 5: B). Exotesta stone cells appear spindle-shaped, rectangular, or polygonal in surface view, with cavities containing brown substances, 12–18 m in diameter, moderately thick walls, and tight arrangement (Figure 5: D). Calcium oxalate cluster crystals measure 30–40 m in diameter (Figure 5: E). Endotesta cells are square or polygonal with beaded wall thickening, 10–15 m in diameter (Figure 5: G). Fibers are slender, fusiform, approximately 20 m in diameter and 400 m in length, often arranged in an interlocking, mosaic pattern (Figure 5: H). Non-glandular hairs are unicellular, long fusiform, slightly swollen at the base, often fragmented, with complete hairs approximately 150 m in length and cavities containing brown substances (Figure 5: J). Spiral vessels are slightly lignified, 12–16 m in diameter and 140–160 m in length (Figure 5: K). Scalariform vessels are slightly lignified, 12–15 m in diameter and 150–160 m in length (Figure 5: L).

Under polarized light, the side view of mesotesta palisade cells, calcium oxalate cluster crystals, and fibers exhibit clear morphological characteristics with colorful luster in the dark field (Figure 5: C, F, I).

2.4 Sequence Information Analysis

The amplification success rate for all four barcode sequences from the four samples in Table 1 was 100%. However, ITS2 sequencing failed for two samples, yielding a 50% sequencing success rate, while psbA-trnH, matK, and rbcL achieved 100% sequencing success. A total of 14 sequences were obtained and analyzed alongside 92 GenBank sequences through multiple sequence alignment (Table 4). Aligned sequence lengths ranged from 248–745 bp, in descending order: matK, rbcL, psbA-trnH, and ITS2. Average GC content ranged from 25.8%–74.4%, in descending order: ITS2, rbcL, matK, and psbA-trnH. The proportion of variable sites, ranging from 9.0%–57.4%, was highest in matK, which also showed the longest sequence length and highest content of both variable and parsimony-informative sites.

2.5 Barcoding Gap Test

A barcoding gap refers to a distinct interval where interspecific genetic variation significantly exceeds intraspecific variation (Meyer et al., 2005). Kimura 2-Parameter (K2P) genetic distances were calculated for all four sequences (Table 5). ITS2 showed intraspecific and interspecific average distances of 0.012 and 0.037, respectively; psbA-trnH showed 0.138 and 0.066; and rbcL showed 0.001 and 0.015. As illustrated in Figure 6 [Figure 6: see original paper], these three barcode sequences exhibited overlap between intra- and interspecific variation without a clear barcoding gap, making them unsuitable for distinguishing Sterculia nobilis from adulterants. In contrast, matK demonstrated an intraspecific average distance of 0.0003 and interspecific average distance of 0.528, with intraspecific variation significantly smaller than interspecific variation and no overlap between the two, forming a distinct barcoding gap that facilitates authentication.

2.6 Cluster Analysis

NJ phylogenetic trees were constructed using MEGA 11 with bootstrap support values (1,000 replicates); values $ $50% are shown. In the ITS2-based tree (Figure 7 [Figure 7: see original paper]: A), sample PP2 clustered with *S. nobilis* sequences in a separate branch, distinguishable from other *Sterculia* species, while sample JPP1 clustered with other *Sterculia* species and could not be distinguished. In the psbA-trnH-based tree (Figure 7: B), all four samples formed a single branch that could not be distinguished from other *Sterculia* species. In the matK-based tree (Figure 7: C), sample JPP1 formed a separate sub-branch with *S. lanceolata* sequences, while other *Sterculia* species formed another branch. In the rbcL-based tree (Figure 7: D), all four samples formed a single branch that could not be distinguished from other *Sterculia* species. These results indicate that ITS2 can effectively distinguish Sterculia nobilis from other *Sterculia* species, matK can effectively differentiate *S. lanceolata* from other *Sterculia* species, while psbA-trnH and rbcL cannot separate Sterculia nobilis or *S. lanceolata* from other congeneric species.

Discussion

3.1 Pharmacognostical Study of Sterculia nobilis Fruit

Macroscopic and microscopic identification methods are crucial for authenticating fruit and seed-derived Chinese medicines. Liang et al. (2021) applied high-resolution digital imaging, large-image stitching, and real-time depth-of-field extension technologies to digitize macroscopic and microscopic characteristics of Chinese medicines, improving authentication accuracy and quality control. Building upon traditional methods and incorporating these technologies, this study obtained holographic color image data for the macroscopic, micro-morphological, and microscopic identification features of Sterculia nobilis fruit. Key diagnostic characteristics were identified: macroscopically, the reddish-brown or dark chestnut seed surface with two thick, light-yellow endosperm lobes; micro-morphologically, the reddish-brown brittle exotesta, black-brown hard mesotesta, and light-yellow soft endotesta. Microscopic powder characteristics identified exotesta stone cells, mesotesta palisade cells, endotesta cells, and calcium oxalate cluster crystals as important diagnostic features, with mesotesta palisade cells and calcium oxalate cluster crystals exhibiting colorful luster under polarized light, enabling rapid identification.

Previous pharmacognostical studies on *S. nobilis* have focused on non-medicinal parts. Su et al. (2019) compared the macroscopic and microscopic characteristics of stems and leaves between *S. nobilis* and *S. lanceolata*, identifying significant differences in leaf, flower, stem transverse section, leaf transverse section, leaf epidermal features, and leaf powder. This study complements previous research by systematically investigating the seeds, which are the medicinal part. During seed transverse section preparation, the large size, multiple cell layers, and loose structure of Sterculia nobilis fruit made traditional hand-sectioning and paraffin sectioning difficult for obtaining complete, thin specimens. Cryosectioning was therefore employed. After fixation and dehydration, samples were embedded and sectioned immediately after rapid freezing, yielding complete 5 m-thick seed transverse sections. This approach avoided sample fragmentation from incomplete paraffin infiltration, simplified the procedure, and produced clear images of complete seed coat structures. This methodology can be applied to other large fruit and seed Chinese medicines.

Sterculia nobilis fruit is known as "Jiucengpi" (nine-layer skin), first mentioned in Wang Ji' s (1936) *Junzitang Rixun Shoujing* from the Ming Dynasty: "In Hengzhou, there is a fruit called Jiucengpi; one must peel nine layers to reach the flesh, which tastes like chestnut when cooked." However, Fan Chengda (1986) in the Song Dynasty described seven layers: "Luohuangzi resembles an olive, with seven layers of skin." These historical records show inconsistent descriptions of seed coat layers, creating confusion for macroscopic identification. Due to the hard texture and difficulty in separating the seed coat layers, the exact number has remained unverified. This study employed cryosectioning and biological microscopy to observe the complete seed coat structure, revealing that Sterculia

nobilis fruit actually has seven distinct layers, providing scientific reference for evaluating the historical nomenclature.

3.2 DNA Barcoding Analysis of Sterculia nobilis Fruit

DNA barcoding offers advantages of accuracy, objectivity, and universality compared to traditional methods and has been applied to various Chinese medicines for phylogenetic studies (Ren et al., 2023), taxonomic identification (Tu and Zhang, 2023), relationship analysis (Wurenji et al., 2022), and genetic diversity assessment (Yin et al., 2023). As DNA barcodes are identical across different tissues from the same plant, sampling difficulty is reduced, and scientific validity is maintained even when different plant parts are collected (Cai et al., 2022; Chen et al., 2021). This study compared ITS2, psbA-trnH, matK, and rbcL sequences from seven *Sterculia* species and conducted cluster analysis to explore their phylogenetic relationships. Results showed that single barcode sequences could not distinguish all seven species but could identify some. ITS2 effectively distinguished Sterculia nobilis from other *Sterculia* species, while matK effectively differentiated *S. lanceolata* from other congeneric species. However, psbA-trnH and rbcL could not separate Sterculia nobilis or *S. lanceolata* from other *Sterculia* species. ITS2 is a commonly used marker for species identification and phylogenetic studies due to its short length, rapid variation at the species level, and numerous mutation sites for species discrimination (Chen et al., 2010). Based on these findings, ITS2 is recommended as the optimal DNA barcode for molecular identification of Sterculia nobilis fruit.

This systematic pharmacognostical study of Sterculia nobilis fruit summarized key macroscopic and microscopic identification features and identified ITS2 as the best DNA barcode for molecular authentication, providing a scientific basis for establishing quality standards and promoting resource development.

References

Cai YM, Dai JP, Zheng YX, et al., 2022. Screening of DNA barcoding sequences for molecular identification of *Uncaria* genus [J]. *Chin Trad Herb Drugs*, 53(6): 1828-1837.

Chen WQ, Wang XF, Chen XY, et al., 2021. Evaluation of the ability of different plant DNA barcodes to identify *Dendrobium officinale* [J]. *Food Sci*, 42(22): 131-139.

Chen SL, Yao H, Han JP, et al., 2013. Principles for molecular identification of traditional Chinese materia medica using DNA barcoding [J]. *Chin J Chin Mater Med*, 38(2): 141-148.

Chinese Materia Medica Editorial Committee of the State Administration of Traditional Chinese Medicine, 1998. *Chinese Materia Medica*: Vol. 5 [M]. Shanghai: Shanghai Scientific and Technical Publishers: 393-394.

Fan CD, 1986. *Guihai Yuheng Zhi* [M]. Nanning: Guangxi People' s Publishing House: 84.

Flora of China Commission, 1984. *Flora of China*: Vol. 49 [M]. Beijing: Science Press: 121.

Jiangsu New Medical College, 1985. *Dictionary of Traditional Chinese Medicine*: Vol. 1 [M]. Shanghai: Shanghai Scientific and Technical Publishers: 490.

Liang ZT, Li XM, Chen LJ, et al., 2021. Application and prospective usage of using digital images to describe morphological features accurately in the authentication of Chinese medicines [J]. *Lishizhen Med Mater Med Res*, 32(3): 631-636.

Ren YY, Zheng Y, Ke JW, et al., 2023. Validation studies of ITS2 sequence as DNA barcode to identify several Saxifragaceae [J]. *Exp Technol Manage*, 40(4): 20-24.

Su YM, Chen SM, Du Q, et al., 2019. Identification of *Sterculia nobilis* Smith and *Sterculia lanceolata* Cav. [J]. *J Guangzhou Univ Trad Chin Med*, 36(3): 414-419.

Tu GZ, Zhang XQ, 2023. Application progress of DNA barcoding technology in *Dendrobium* classification and identification [J]. *J Food Saf Qual*, 14(2): 154-160.

Wang DM, 2002. Tropical precious dried fruit—Sterculia nobilis fruit [J]. *Yunnan For*, 23(1): 18.

Wang J, 1936. *Junzitang Rixun Shoujing* [M]. Beijing: The Commercial Press: 14.

Wurenji RL, Jin SJ, Wu YH, et al., 2022. Identification of medicinal *Aconitum* species based on ITS2 sequences and analysis of genetic relationship [J]. *Chin J Exp Trad Med Form*, 28(17): 157-163.

Yin GY, Yuan L, Wang X, et al., 2023. Screening of specific DNA barcode, identification of germplasm resources, and analysis of genetic diversity of *Atractylodes chinensis* [J]. *Acta Pharm Sin*, 58(6): 1693-1704.

Chen SL, Yao H, Han JP, et al., 2010. Validation of the ITS2 region as a novel DNA barcode for identifying medicinal plant species [J]. *PLoS ONE*, 5(1): e8613.

Meyer CP, Paulay G, 2005. DNA barcoding: error rates based on comprehensive sampling [J]. *PLoS Biol*, 3(12): 2229-2238.

Zhang JJ, Li Y, Lin SJ, et al., 2018. Green extraction of natural antioxidants from the *Sterculia nobilis* fruit waste and analysis of phenolic profile [J]. *Molecules*, 23(5): 1059-1072.

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