
Identification of Three Chinese Herbal Medicines and Their Adulterants Based on Allele-Specific Diagnostic PCR

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To cite this article:

Hao Chen, Xiao-Lei Chen, Jian-Yun Su, Yan-Ying Li, Xiao-Bin Hu, Zhe Xu, Hua-Li Qian, Lei Zhang, Jia-Hong Dong, Peng-Zhang Ji. Identification of Three Chinese Herbal Medicines and Their Adulterants Based on Allele-Specific Diagnostic PCR. *Journal of Plant Sciences*. Vol. 10, No. 6, 2022, pp. 214-221. doi: 10.11648/j.jps.20221006.13

Received: October 14, 2022; **Accepted:** November 19, 2022; **Published:** November 29, 2022

Abstract: Owing to the high pharmacological and economic value, the Chinese medicinal herbs: *Paris polyphylla* var. *yunnanensis*, *Polygonatum kingianum* and *Bletilla striata*, whose quality has been damaged by their adulterants. In this study, the allele-specific diagnostic PCR method was used to identify three herbal medicines and their adulterants. And the nuclear gene ITS2 sequence and chloroplast gene psbA-trnH sequence, as international common barcode sequences, are widely used in the molecular identification of medicinal plants. Therefore, three pair of specific PCR primers were respectively designed according to the sequence of *Paris polyphylla* var. *yunnanensis* on the ITS2, *Polygonatum kingianum* and *Bletilla striata* on the psbA-trnH, using Primer 5.0. And these specific identification primers could amplify special bands respectively at 130bp, 331bp and 508bp, while adulterants could not amplify the bands. The results showed that this method could respectively distinguish three herbal medicines and their adulterants. And after many experiments, the method had good repeatability. The allele-specific PCR identification method established in this study is easy to operate and fast in identification. This method can distinguish three kinds of traditional Chinese medicines and their adulterants, which provides a reference for the identification of other traditional Chinese medicines, so as to further protect the Chinese herbal medicine industry from economically motivated adulteration.

Keywords: Paris Polyphylla var. Yunnanensis, Polygonatum Kingianum, Bletilla Striata, Adulterants, Allele-Specific Diagnostic PCR, Identification

1. Introduction

At present, in the actual production and marketing of Chinese herbal medicines, many economically motivated adulterations have occurred. Traditional methods for identification of medicinal herbs rely mainly on morphological and histological characteristics. However, most traditional Chinese medicinal materials are very similar to their original plants, and are difficult to be identified. Moreover, traditional morphological and histological identification methods are susceptible to human factors and environmental factors, and inevitably exist errors. Therefore,

finding an accurate identification method becomes the key to solve the problem. In recent years, with the development of molecular identification technology, more and more researchers have applied molecular identification technology in the authentication of Chinese herbal medicines. Nevertheless, currently reported molecular identification methods are often complex and time-consuming, such as using cpDNA rbcL and translated amino acid sequences to distinguish *Dryopteris crassirhizoma* and adulterant species [1], using DNA barcodes for the identification of *Anoectochilus roxburghii* and its adulterants [2], using species-unique single nucleotide polymorphism markers to develop an authentication system of *Panax* [3]. Allele-specific

PCR only needs a PCR reaction, and then we can judge the authenticity from whether there is an amplification, which is simple and fast. Nowadays, the allele-specific PCR technology has been applied in *Panax ginseng* [4, 5], *Humulus lupulus* L. [6], *Sarcandra glabra* and *Chloranthus* [7], *Amomum villosum* Lour. [8], *Mentha haplocalyx* and *Mentha spicata* [9], *Pericarpium citri reticulatae* and *Citriunshius pericarpium* [10], *Ligusticum tenuissimum* [11].

P. polyphylla var. *yunnanensis* is one of original plants of Chinese medicinal "Chong Lou". *P. kingianum* is one of original plants of Chinese medicinal "Huang Jing". And *B. striata* is the original plant of Chinese medicinal "Bai Ji" [12]. The rhizomes of them are used as Chinese herbal pieces. They all are perennial herbs, among them, *P. polyphylla* var. *yunnanensis*, is mainly distributed in southwest China. *P. kingianum* is mainly distributed in Yunnan, Sichuan, Guizhou and Guangxi provinces of China. And *B. striata* is found mainly in China, Japan, and northern Myanmar [13]. *P. polyphylla* var. *yunnanensis* is widely reported to have antibacterial activity [14], cytotoxic [15], antioxidant activity [16], antitumor activity [17]. *P. kingianum* is widely used to treat hyperlipidemia [18], nonalcoholic fatty liver [19] and diabetes [20]. And *B. striata* is widely used to treat enterocutaneous fistula [21], ulcerative colitis [22] and bleeding [23] in East Asian countries.

The available supply of these herbs is facing an increasing shortage because its growth period needs more than 3 years,

and the consumption of these herbs in the pharmaceutical industry has increased dramatically in recent years. The price of *P. polyphylla* var. *yunnanensis* exceeded 800 yuan/kg in 2018, so its mixed adulterants appeared in the sales market, and the same as *P. kingianum* and *B. striata*. The adulterants have affected the quality of three Chinese herbal medicines seriously. In this study, our aim is to use the allele-specific diagnostic PCR of ITS2 and non-coding region of chloroplast gene to identify *P. polyphylla* var. *yunnanensis*, *P. kingianum* and *B. striata*, and their adulterants respectively.

2. Materials and Methods

2.1. Plant Materials and Genomic DNA Extraction

There are three groups of materials, *P. polyphylla* var. *yunnanensis* and its adulterants (Table 1); *P. kingianum* and its adulterants (Table 2); *B. striata* and its adulterants (Table 3). Samples were collected from Yunnan province, China, with the exception of *Solanum tuberosum*, *Ipomoea batatas*, *Dioscorea polystachya* Turcz, *Zingiber officinale* and *Colocasia esculenta* purchased from the local market. Genomic DNA was extracted from leaves or roots using the improved CTAB method, dissolved in sterile ddH₂O, and stored at -20°C. The quality and the concentration of extracted genomic DNA were measured using an ultraviolet spectrophotometer and agarose gel electrophoresis.

Table 1. Samples of *P. polyphylla* var. *yunnanensis* and its adulterants used in this study.

Chinese name	Amounts	Location	Scientific name
Dian Chong Lou	13	Kunming, Yunnan, China	<i>P. polyphylla</i> var. <i>yunnanensis</i>
Mao Chong Lou	7	Wenshan, Yunnan, China	<i>Paris mairei</i>
Hua Ye Chong Lou	9	Kunming, Yunnan, China	<i>Paris marmorata</i>
Xia Ye Chong Lou	6	Wenshan, Yunnan, China	<i>Paris polyphylla</i> var. <i>stenophylla</i>
Da Li Chong Lou	17	Wenshan, Yunnan, China	<i>Paris daliensis</i>
Nan Chong Lou	20	Wenshan, Yunnan, China	<i>Paris vietnamensis</i>
Chang Zhu Chong Lou	11	Kunming, Yunnan, China	<i>Paris forrestii</i>
Qi Ye Yi Zhi Hua	15	Kunming, Yunnan, China	<i>Paris polyphylla</i>
Wu Zhi Lian Chong Lou	4	Kunming, Yunnan, China	<i>Paris axialis</i>

Table 2. Samples of *P. kingianum* and its adulterants used in this study.

Chinese name	Amounts	Location	Scientific name
Dian Huang Jing	21	Yanjin, Yunnan, China	<i>P. kingianum</i>
Yu Zhu	7	Kunming, Yunnan, China	<i>Polygonatum odoratum</i>
Ku Huang Jing	5	Kunming, Yunnan, China	Unidentify
Xiao Huang Jing	8	Kunming, Yunnan, China	<i>Polygonatum uncinatum</i> Diels
Yang Yu	6	Kunming, Yunnan, China	<i>Solanum tuberosum</i>
Fan Shu	6	Kunming, Yunnan, China	<i>Ipomoea batatas</i>
Shu Yu	6	Kunming, Yunnan, China	<i>Dioscorea polystachya</i> Turcz.
Jiang	6	Kunming, Yunnan, China	<i>Zingiber officinale</i>
Yu	6	Kunming, Yunnan, China	<i>Colocasia esculenta</i>

Table 3. Samples of *B. striata* and its adulterants used in this study.

Chinese name	Amounts	Location	Scientific name
Bai Ji	30	Kunming, Yunnan, China	<i>B. striata</i>
Shan Lan	20	Wenshan, Yunnan, China	<i>Oreorchis patens</i>
Jian Xue Qing	20	Kunming, Yunnan, China	<i>Liparis nervosa</i>
Du Suan Lan	11	Wenshan, Yunnan, China	<i>Pleione bulbocodioides</i>
Tong Ban Lan	14	Wenshan, Yunnan, China	<i>Anthogonium gracile</i>
Yue Nan Bai Ji	7	Wenshan, Yunnan, China	Unidentify

2.2. PCR amplification Conditions

The total volume of the PCR reaction system was 25 μ L, the reaction system including 2.5 μ L 10 * PCR Taq buffer (Mg²⁺ plus 20mM), 0.5 μ L (10mM) dNTP, 1 μ L (10 μ M) of each primer, 0.5 μ L (2U/ μ L) DNA Taq polymerase (Dingguo Changsheng, Beijing, China), 1 μ L (10-100ng) DNA template and 18.5 μ L ddH₂O. And the amplification of universal primers were amplified by PreMix (2 \times PCR Master Mix, SangonBiotech, Shanghai, China). The PCR cycling program included an initial step at 94°C for 5 min followed by 35 cycles at 94°C for 1min, at 56°C for 1min, and at 72°C for 1min, and a final extension at 72°C for 7 min. Finally, PCR products were examined on 1.5% agarose gels. The universal primers ITS2-F and ITS2-R were used to amplify *P. polyphylla* var. *yunnanensis* and its adulterants (Figure 1). The universal primers psbA-trnH-F and psbA-trnH-R were not only used to amplify *P. kingianum* and its adulterants (Figure 2), but also used to amplify *B. striata* and its adulterants (Figure 3).

2.3. Design of Identification Primer

BioEdit software was used for sequence analysis, collation and comparison, to find out the specific mutation sites of the samples (*P. polyphylla* var. *yunnanensis*; *P. kingianum*; *B. striata*). And Premier 5.0 was used to design three pairs of specific PCR primers (DCL-F, DCL-R; DHJ-F, DHJ-R; BJ-F, BJ-R). (Tables 4-6)

2.4. Specific PCR Reaction Conditions

The following reaction procedure can effectively distinguish the above mentioned authentic Chinese herbal medicine from its adulterants. After repeated tests, the effect is ideal. And the samples that participated in the amplification reaction were *P. polyphylla* var. *yunnanensis* and its adulterants (pre-denaturation at 94°C for 3 min, denaturation at 94°C for 30s, annealing at 56°C for 45s, refolding at 72°C for 1 min, extension at 72°C for 10 min, at 4°C for storage, 35 cycles) (Figure 4); *P. kingianum* and its adulterants (pre-denaturation at 94°C for 4 min, denaturation at 94°C for 1min, annealing at 56°C for 45s, refolding at 72°C for 1 min, extension at 72°C for 10 min, at 4°C for storage, 32 cycles) (Figure 5); *B. striata* and its adulterants (pre-denaturation at 94°C for 4 min, denaturation at 94°C for 45s, annealing at 58°C for 1 min, refolding at 72°C for 1 min, extension at 72°C for 7 min, at 4°C for storage, 35 cycles) (Figure 6).

3. Results

3.1. Assay of Universal Primer Amplification

Select universal primers (ITS2; psbA-trnH) to amplify authentic herbs (*P. polyphylla* var. *yunnanensis*; *P. kingianum*; *B. striata*) and their adulterants, and conduct electrophoresis detection, as shown in Figures 1-3.

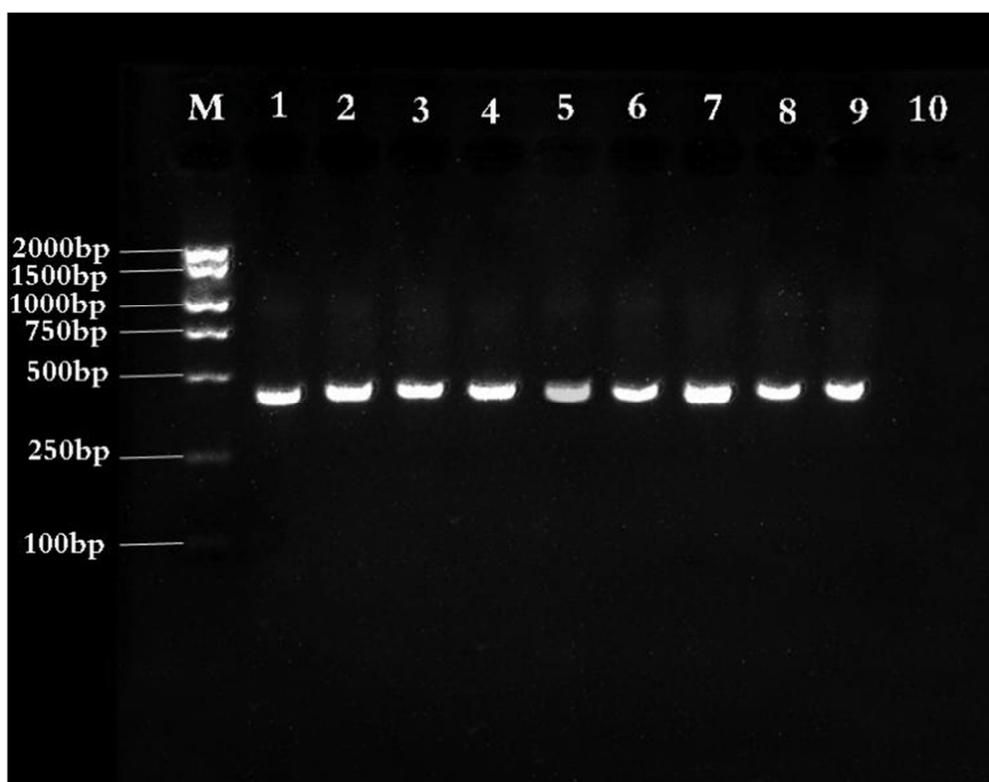


Figure 1. Gel electrophoresis of ITS2 amplification for *P. polyphylla* var. *yunnanensis* and its adulterants. (1. *P. polyphylla* var. *yunnanensis*. 2. *Paris mairei*. 3. *Paris marmorata*. 4. *Paris polyphylla* var. *stenophylla*. 5. *Paris daliensis*. 6. *Paris vietnamensis*. 7. *Paris forrestii*. 8. *Paris polyphylla*. 9. *Paris axialis*. 10. Negative control.).

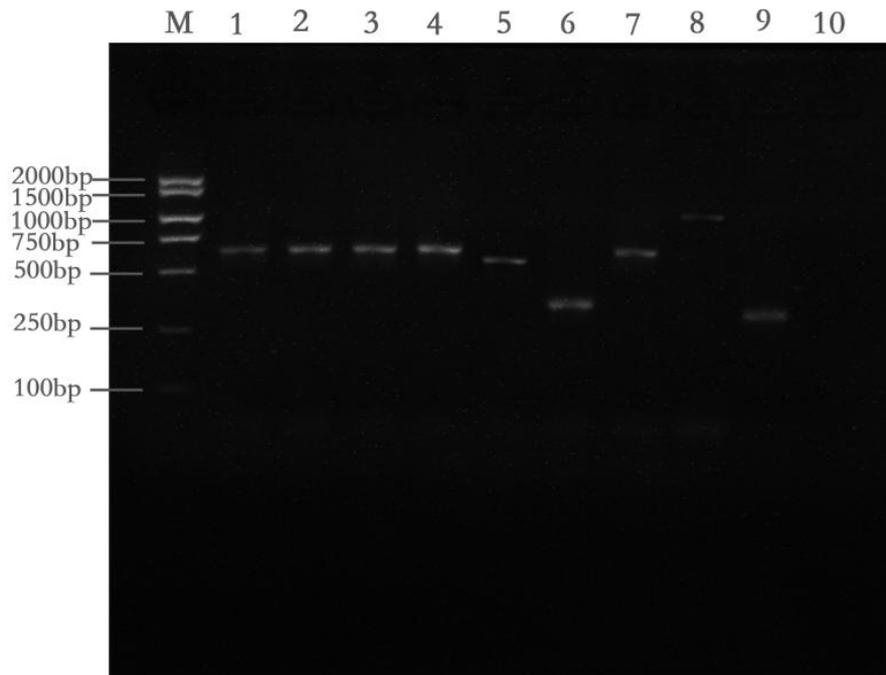


Figure 2. Gel electrophoresis of *psbA-trnH* amplification for *P. kingianum* and its adulterants. (1. *P. kingianum* 2. *P. odoratum*. 3. *Ku Huang Jing*. 4. *P. uncinatum* Diels. 5. *Solanum tuberosum*. 6. *Ipomoea batatas*. 7. *Dioscorea polystachya* Turcz. 8. *Zingiber officinale* 9. *Colocasia esculenta*. 10. Negative control.).

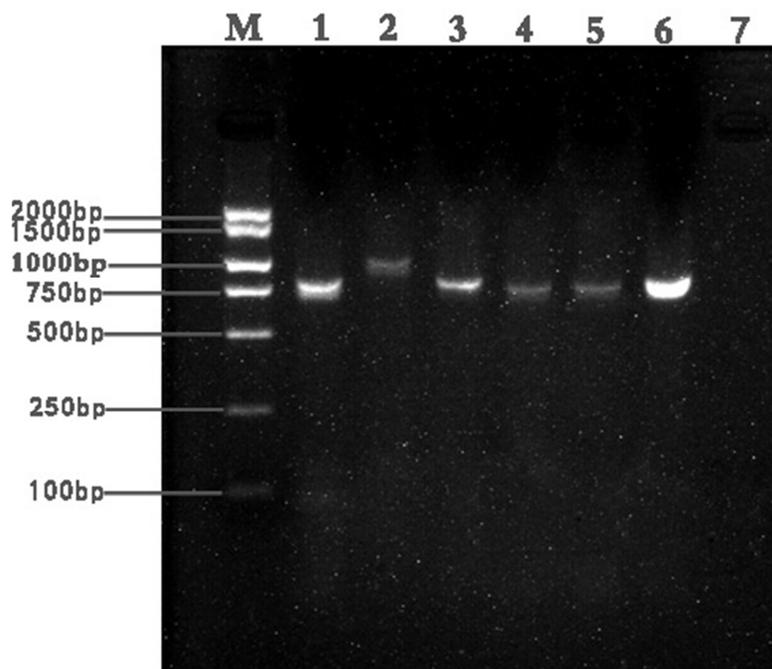


Figure 3. Gel electrophoresis of *psbA-trnH* amplification for *B. striata* and its adulterants. (1. *B. striata*. 2. *Oreorchis patens*. 3. *Liparis nervosa*. 4. *Pleione bulbocodioides*. 5. *Anthogonium gracile*. 6. *Yue Nan Bai Ji*. 7. Negative control.).

3.2. Specific Primer Design

The primers were used to amplify the samples, and the amplified products were sent to Sangon Biotech Company for sequencing. GenBank was used to search the identification primers for authentic herbs and their adulterants. The sequencing results were analyzed and compared by BioEdit software to find out the unique mutation sites of authentic

herbs. And three pairs of specific PCR primers (*P. polyphylla* var. *yunnanensis*: DCL-F, DCL-R; *P. kingianum*: DHJ-F, DHJ-R; *B. striata*: BJ-F, BJ-R) were designed using Primer Premier 5.0 software. The nucleic acid sequence is shown in Tables 4-6.

The mutation sites of *P. polyphylla* var. *yunnanensis* are numerous, it is A in 190bp, *Paris polyphylla* var. *stenophylla* is T in 190bp, while others are C; it is C in

203bp while others are T; it is G in 206bp while others are C; it is A in 275bp while others are C; it is G in 276bp while others are C; it is A in 320bp, others are G; it is A in 331bp, others are T; it is A in 363bp, others are C; it is T in 389bp, others are C; it is T in 391bp, others are C, and specific primers (DCL-F/R) are designed with 203 bp and 331 bp

loci, respectively. The mutation site of *P. kingianum* in 71bp is A, and *Polygonatum odoratum* and *Polygonatum uncinatum* Diels are T, while others are none; it is C in 402bp, while Ku Huang Jing is A, and *Colocasia esculenta* is T. The mutation site of *B. striata* in 111bp is A, and *Oreorchis patens* is C, while others are T.

Table 4. *P. polyphylla* var. *yunnanensis* primers and PCR reaction conditions used in this study.

Primer name	Sequence	Reaction condition
ITS2-F	5'-ATGCGATACTTGGTGTGAAT-3'	94°C5mins, 94°C30s, 56°C30s, 72°C45s, 40cycles, 72°C7mins,
ITS2-R	5'-GACGCTTCTCCAGACTACAAT-3'	
DCL-F	5'-CGCGTACAACGAAACCATGCTGGGC-3'	94°C3mins, 94°C30s, 56°C45s, 72°C1mins, 35cycles, 72°C10mins,
DCL-R	5'-GAAGAGTGGGATGCCAACTGAGACC-3'	

Table 5. *P. kingianum* primers and PCR reaction conditions used in this study.

Primer name	Sequence	Reaction condition
psbA-trnH-F	5'-GTTATGCATGAACGTAATGCTC-3'	94°C5mins, 94°C1min, 56°C1min, 72°C1min, 32cycles, 72°C7mins
psbA-trnH-R	5'-CGCGCATGGTGGATTCACAATCC-3'	
DHJ-F	5'-ATGTATTAAGAATCGTTGAAGGAGC-3'	94°C4mins, 94°C1min, 56°C45s, 72°C1min, 32cycles, 72°C10mins
DHJ-R	5'-AGCTAATCATTATCGAGAAAAATG-3'	

Table 6. *B. striata* primers and PCR reaction conditions used in this study.

Primer name	Sequence	Reaction condition
psbA-trnH-F	5'-GTTATGCATGAACGTAATGCTC-3'	94°C5mins, 94°C1min, 56°C1min, 72°C1min, 32cycles, 72°C7mins,
psbA-trnH-R	5'-CGCGCATGGTGGATTCACAATCC-3'	
BJ-F	5'-AGCAATCCCCCAATATCTTGTCTA-3'	94°C4mins, 94°C45s, 58°C1mins, 72°C1min, 32cycles, 72°C7mins,
BJ-R	5'-GGTCTCGGGCATCTACCA-3'	

3.3. Specific Primer Verification

Finally, the samples were amplified by PCR with specific primers to verify the identification effect. Put 5µL PCR amplification products into 1.5% agarose gel containing nucleic acid dyes, and electrophoresis at 100V for 40 minutes.

Results as shown in Figures 4-6, authentic Chinese herbal medicine can obtain PCR product bands of different lengths, while other adulterants cannot produce bands. The samples identified at 130bp were all *P. polyphylla* var. *yunnanensis*, and at 331bp were all *P. kingianum*, and at 508bp were all *B. striata*.

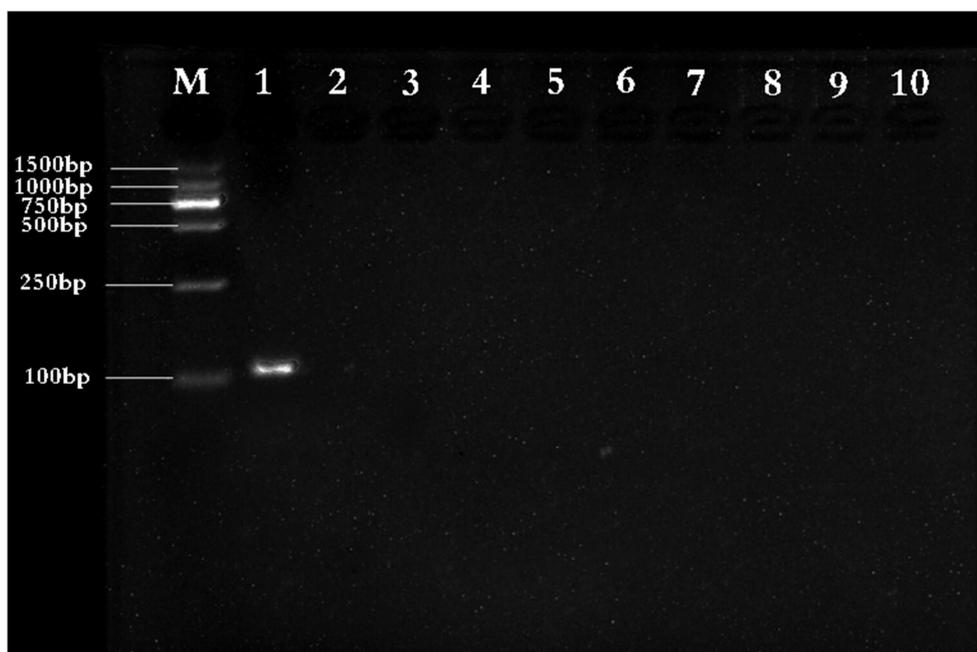


Figure 4. Gel electrophoresis of specific primer amplification for *P. polyphylla* var. *yunnanensis* and its adulterants. (1. *P. polyphylla* var. *yunnanensis*. 2. *Paris mairei*. 3. *Paris marmorata*. 4. *Paris polyphylla* var. *stenophylla*. 5. *Paris daliensis*. 6. *Paris vietnamensis*. 7. *Paris forrestii*. 8. *Paris polyphylla*. 9. *Paris axialis*. 10. Negative control.).

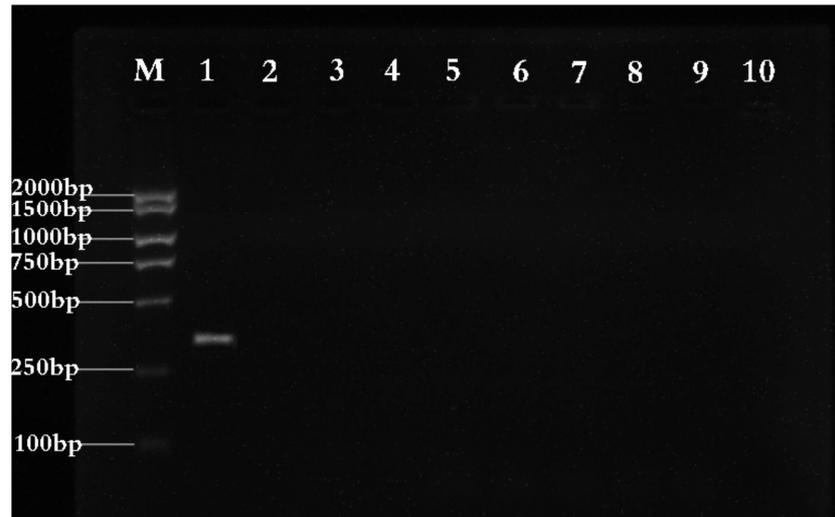


Figure 5. Gel electrophoresis of specific primer amplification for *P. kingianum* and its adulterants. (1. *P. kingianum*. 2. *P. odoratum*. 3. *Ku Huang Jing*. 4. *P. uncinatum* Diels. 5. *Solanum tuberosum*. 6. *Ipomoea batatas*. 7. *Dioscorea polystachya* Turcz. 8. *Zingiber officinale* 9. *Colocasia esculenta* 10. Negative control.).

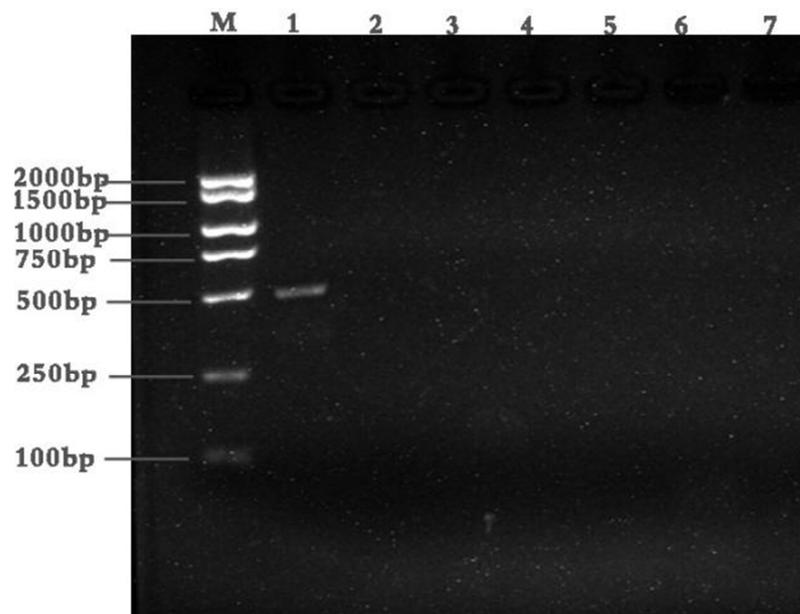


Figure 6. Gel electrophoresis of specific primer amplification for *B. striata* and its adulterants. (1. *B. striata* 2. *Oreorchis patens* 3. *Liparis nervosa*. 4. *Pleione bulbocodioides*. 5. *Anthogonium gracile*. 6. *Yue Nan Bai Ji*. 7. Negative control.).

4. Discussion

The identification of plant species is very significant for the standardization of the herbal medicine industry and the prevention of economically motivated adulteration. Because some Chinese herbal medicines have high pharmacological and economic value, there is ample potential for economically motivated adulteration of Chinese herbal medicine products. Therefore, a simple, reliable and practical method for accurately identifying the authenticity of Chinese herbal medicine plays an important role in the development and protection of the Chinese herbal medicine industry.

There are many identification methods for Chinese medicinal materials, including original plant identification, character identification, microscopic identification and

physical and chemical identification, but these identification methods are often affected by human or environmental factors. In contrast, molecular identification technology is stable and not affected by human or environmental factors. Allele specific PCR means that the base mismatch between primer and template can effectively inhibit the PCR reaction, and then achieve the purpose of template differentiation (allelic differentiation). The allele specific PCR is a new technique that is widely used as a biological tool for species identification, breeding, and evolutionary research.

ITS2 sequence is located between 5.8S and 28S nuclear rDNA genes. In plant cells, the secondary structure of ITS2 rDNA is a stem loop structure formed by the self-folding of the primary sequence to form partial base pairing and alternating single strand. The secondary structure of eukaryotic ITS2 is a highly conservative "one ring four arm"

model, which is short in length and easy to amplify, and is suitable for the identification of plant genera and species [24]. The psbA trnH sequence is the spacer sequence between the psbA gene (encoding the D1 protein of the photosynthetic system II reaction center) and the trnH gene (encoding tRNA histidine) in chloroplasts. This sequence only plays the role of linking two genes. It has low selection pressure, relatively many mutant sites, fast evolution rate, and conservative DNA sequences at both ends, facilitating the design of universal primers [25]. Therefore, the nuclear gene ITS2 sequence and chloroplast gene psbA-trnH sequence, as international common barcode sequences, are widely used in the molecular identification of medicinal plants.

5. Conclusion

In this study, based on the original plant ITS2 sequence and psbA-trnH sequence, site specific primers were screened to identify *P. polyphylla* var. *yunnanensis*, *P. kingianum* and *B. striata*, and their adulterants, and a allele specific PCR method was established. By optimizing the reaction conditions, stable and clear identification bands were obtained using this method, which can accurately identify the three kinds of Chinese medicines and their adulterants. The establishment of this method is different from DNA sequence analysis method. The allele specific PCR identification method established in this study is easy to operate and fast in identification. It does not require high technical level for identification personnel and has high application value. This study has successfully identified three kinds of traditional Chinese medicines and their adulterants by site specific PCR, which provides a reference for the identification of the authenticity of traditional Chinese medicines in the future.

Acknowledgements

This work was supported by the Natural Science Foundation of China (31760347), the Joint Special Program of Chinese Medicine of the Natural Science Foundation of Yunnan Province (202001AZ070001-010), the Innovative Talents Foundation of Yunnan Province (2018HB085), and the high level talents Foundation of Yunnan University of Chinese Medicine and Innovation Team of Yunnan Colleges and Universities (30270104868; 20270101831).

References

- [1] Zl, Zhao, Leng Ch and Wang Zt. "Identification of *Dryopteris crassirhizoma* and the adulterant species based on cpDNA rbcL and translated amino acid sequences." *Planta Medica* 73 (2007): 1230-1233.
- [2] Lv, Tongwei, Renda Teng, Qingsong Shao, Hongzheng Wang, Wangshu Zhang, Ming-yan Li and Lili Zhang. "DNA barcodes for the identification of *Anoectochilus roxburghii* and its adulterants." *Planta* 242 (2015): 1167-1174.
- [3] Nguyen, Van Binh, Vo Ngoc Linh Giang, Nomar Espinosa Waminal, Hyun-Seung Park, Nam-Hoon Kim, Woojong Jang, Junki Lee and Tae-Jin Yang. "Comprehensive comparative analysis of chloroplast genomes from seven *Panax* species and development of an authentication system based on species-unique single nucleotide polymorphism markers." *Journal of Ginseng Research* 44 (2020): 135-144.
- [4] Wang, Hongtao, Gui-sheng Li, Woo Saeng Kwon and Deok-Chun Yang. "Development of EST Intron-Targeting SNP Markers for *Panax ginseng* and Their Application to Cultivar Authentication." *International Journal of Molecular Sciences* 17 (2016): 884-895.
- [5] Wang, Hongtao, Hua Sun, Woo Saeng Kwon, Haizhu Jin and Deok-Chun Yang. "A PCR-based SNP marker for specific authentication of Korean ginseng (*panax ginseng*) cultivar "Chunpoong"." *Molecular Biology Reports* 37 (2009): 1053-1057.
- [6] Henning, John A., J. Coggins and Matthew Peterson. "Simple SNP-based minimal marker genotyping for *Humulus lupulus* L. identification and variety validation." *BMC Research Notes* 8 (2015): 542-553.
- [7] Wei, Yi-cong, Ying Chen, Youkai Huang, Jinping Liu and Yi-chi Liang. "Molecular authentication and quantitative analysis of *Sarcandra glabra* and adulterated chloranthus products using SNP markers." *Mitochondrial DNA Part A* 27 (2016): 3618-3625.
- [8] Huang, Qionglin, Zhonggang Duan, Jin-Fen Yang, Xinye Ma, Ruoting Zhan, Hui Xu and Weiwen Chen. "SNP Typing for Germplasm Identification of *Amomum villosum* Lour. Based on DNA Barcoding Markers." *PLoS ONE* 9 (2014): e114940.
- [9] Cao, Liang, Shuang-shuang Qin, Yuan Yuan and Xiao-qi Zhu. "Molecular identification of *Mentha haplocalyx* and *Mentha spicata* with specific primers multi-PCR system." *Zhong yao cai = Zhongyao cai = Journal of Chinese medicinal materials* 37 1 (2014): 41-5.
- [10] Wang, Hongtao, Min-Kyeoung Kim, Yeon Ju Kim, Hee Lee, Haizhu Jin, Jiayu Chen and Deok-Chun Yang. "Molecular authentication of the Oriental medicines *Pericarpium citri reticulatae* and *Citri unshius pericarpium* using SNP markers." *Gene* 494 1 (2012): 92-5.
- [11] Jigden, Baigalmaa, Hongtao Wang, Myung Kyum Kim, Yeon Ju Kim, Jun Gyo In and Deok-Chun Yang. "Authentication of the oriental medicinal plant *Ligusticum tenuissimum* (Nakai) Kitagawa (Korean Go-Bon) by multiplex PCR." *Planta medica* 76 6 (2010): 648-51.
- [12] Chinese Pharmacopoeia Commission, China Medical Science Press, Pharmacopoeia of the People's Republic of China, 2015, pp. 306.
- [13] Chinese Flora Commission, Science Press and Missouri Botanical Garden Press, Flora of China, 2000, pp. 24.
- [14] Qin, Xu-Jie, Dongyang Sun, Wei Ni, Chang-Xiang Chen, Yan Hua, Li He and Haiyang Liu. "Steroidal saponins with antimicrobial activity from stems and leaves of *Paris polyphylla* var. *yunnanensis*." *Steroids* 77 (2012): 1242-1248.
- [15] Wang, Yue-Hu, Min Shi, Hong-mei Niu, Jun Yang, Meng-yuan Xia, Ji-Feng Luo, Yingjie Chen, Yi-ping Zhou and Heng Li. "Substituting one *Paris* for another in vitro cytotoxic and in vivo antitumor activities of *Paris forrestii*, a substitute of *Paris polyphylla* var. *yunnanensis*." *Journal of ethnopharmacology* 218 (2018): 45-50.

- [16] Men, Wei. "Extraction and Antioxidant Activity of Total Saponins from Stems and Leaves of *Paris polyphylla* var. *yunnanensis* (Franch.) Hand.-Mazz." *Natural Product Research and Development* (2015): 347-352.
- [17] Jing, Songsong, Ying Wang, Xia Li, Shuli Man and Wenyuan Gao. "Chemical constituents and antitumor activity from *Paris polyphylla* Smith var. *yunnanensis*." *Natural Product Research* 31 (2017): 660-666.
- [18] Gu, Wen, Yan-fang Wang, Linxi Zeng, Jincai Dong, Qian Bi, Xingxin Yang, Yanyun Che, Sen He and Jie Yu. "Polysaccharides from *Polygonatum kingianum* improve glucose and lipid metabolism in rats fed a high fat diet." *Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie* 125 (2020): 109.
- [19] Yang, Xingxin, Xi Wang, Tingting Shi, Jincai Dong, Feng-Jiao Li, Linxi Zeng, Min Yang, Wen Gu, Jingping Li and Jie Yu. "Mitochondrial dysfunction in high-fat diet-induced nonalcoholic fatty liver disease: The alleviating effect and its mechanism of *Polygonatum kingianum*." *Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie* 117 (2019): 109083.
- [20] Lu, Jian-mei, Yan-fang Wang, Hong-li Yan, Pei Lin, Wen Gu and Jie Yu. "Antidiabetic effect of total saponins from *Polygonatum kingianum* in streptozotocin-induced diabetic rats." *Journal of ethnopharmacology* 179 (2016): 291-300.
- [21] Zhou, Hong, Yan Jin, Chen Gu, Yigang Chen and Jiazeng Xia. "*Bletilla striata* promotes the healing of enterocutaneous fistula." *Medicine* 98 (2019): e16288.
- [22] Junfeng, Zhang. "*Bletilla striata* polysaccharide for the treatment of ulcerative colitis in mice." *Journal of China Pharmaceutical University* (2012): 535-540.
- [23] Zhai, Wanchen, Enwei Wei, Rui Li, Tianyi Ji, Yueyao Jiang, Xiaoxiao Wang, Yiyang Liu, Zhiying Ding and Hongli Zhou. "Characterization and Evaluation of the Pro-Coagulant and Immunomodulatory Activities of Polysaccharides from *Bletilla striata*." *ACS Omega* 6 (2021): 656-665.
- [24] Chen, Shilin, Hui Yao, Jianping Han, Chang Liu, Jingyuan Song, Linchun Shi, Ying-jie Zhu, Xinye Ma, Ting Gao, Xiaohui Pang, Kun Luo, Y. Li, Xiwen Li, Xiaocheng Jia, Yu-lin Lin and Christine J. Leon. "Validation of the ITS2 Region as a Novel DNA Barcode for Identifying Medicinal Plant Species." *PLoS ONE* 5 (2010): e8613.
- [25] Shaw, Joey, Edgar B. Lickey, J. Thomas Beck, Susan Beth Farmer, Wusheng Liu, Jermy Miller, Kunsiri Chaw Siripun, Charles Thomas Winder, Edward Schilling and Randall L. Small. "The tortoise and the hare II: relative utility of 21 noncoding chloroplast DNA sequences for phylogenetic analysis." *American journal of botany* 92 1 (2005): 142-66.