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New Aspects in Chinese Herb Materials Identification by DNA Barcoding

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ABSTRACT

The numerous noxious reactions have overwhelming concerning by misusing medicinal plant ingredients. This phenomenon has aroused the worldwide demand over the safe application in pharmaceuticals. DNA barcoding offers a powerful means to complement morphological and chemical processes for distinguishing Chinese medicinal plants. Consequently, a DNA barcoding system should be continuous renewal containing mass information about authentic plant materials and potentially substitutes or adulterants. Recent accomplishments in the new field of DNA barcoding that provide new aspects for identification Chinese herbal medicines. Several DNA regions (ITS, ITS2, psbA-trnH, rbcL, matK) have been established as the hot topical barcodes for Chinese herb. This review summarizes the significant progresses in the DNA barcoding about Chinese herb material authentication.

INTRODUCTION

Herbal medicines have been used worldwide for healing and curing disease, which have a long and well-documented history. The World Health documented that 80% of the global population utilizes traditional Chinese medicines for important medicinal properties and healthcare efficacies (<http://www.worldwildlife.org/what/globalmarkets/wildlifetrade/faqs-medicinalplant.html>). From time to time, adulterants and counterfeits of herbal medicines present a primary concern of users, patients and industry as a result of safety and efficacy. Traditional methods to authenticate herbal materials using morphological, microscopic and chemical methods, and they continue to provide the main methods used in pharmacopoeia. However, these techniques have their limitations. Such as traditional taxonomic identification of species usually require the expertise with long training and experience. The adulteration of herbal medicinal materials in the growing herbal market has grown as a global problem. Using the Aristolochia herbs as an example, the family *Aristolochiaceae* species shared commonly adulterants and semblable morphological features in traditional Chinese medicines, which increased the risk of confusion amongst consumers^[1,2]. *Akebiae caulis* derived from *Akebia quinata*, *Ak. trifoliata* or *Ak. trifoliata* var. *australis* (Mutong, MT) was always replaced with *Ar. manshuriensis* (Guanmutong, GMT). *Caulis Aristolochiae Manshuriensis*. Includes aristolochic acids (AA), a nitrophenanthrene carboxylic acid derived from Aristolochia species, which can cause nephropathy^[3]. Aristolochic acid nephropathy (AAN) is a speedy progressive renal interstitial fibrosis resulting in end-stage renal failure disease and urothelial malignancies, was first reported in Belgium in 1991 that more than 100 patients after taking dietary supplement containing a Chinese herb *Stephaniae Tetrandrae Radix* (Fangji, FJ) derived from *Stephania tetrandra* was in advertently substituted with a rich in AA-containing herb derived from *Aristolochia fangchi* (Guangfangji, GFJ)^[4]. Subsequently, the herb adulteration of Aristolochia species have been a worldwide problem and the Food and Drug Administration has warned the safety of botanical products and dietary remedies containing AA^[5]. However, many Aristolochia species are still most common used in Asia and Americas for medicinal purposes^[6]. Remarkably, the adverse reaction due to the misuse, misidentification and adulteration in the herbs containing AA can lead to DNA molecules nucleotide AT-TA transversion mutation, which bind

to mutagenic AA [3,7]. Hence, an accurate, rapid and stable particular marker permitting non-specialists to identify herbal medicinal materials from a standard DNA sequence is undoubtedly beneficial.

The DNA barcode is a relatively new concept for providing rapid, accurate and automatable species identification using a short DNA segments from part of genome. The concept was first started with the seed plants of Hebert [8], who demonstrated that it could be acquire 100% accuracy identification in 200 closely related species of lepidopterans using the mitochondrial gene cytochrome C oxidase subunit I(COI). But the portion of the mitochondrial gene COI will be a more challenging task due to a much slower rate of sequence evolution in higher plants than in those observed in animal mitochondria. An ideal DNA barcode for land plants should be satisfied with three criteria: (i) contain maximal species-level genetic changeability and divergence; (ii) an appropriately conserved flanking sites so as to develop universal primers for bioinformatics analysis and application; (iii) possess an enough short DNA sequence in order to facilitate DNA extraction and PCR amplification. Hence, at present there is no standard protocol for DNA barcoding in land plants.

First, most mitochondrial genes, as well as single-copy genes in the nuclear genome, have been removed as barcode candidates because of the low rate of sequence change and the insufficiency of universal primers for PCR amplification [9]. However, the internal transcribed spacer (ITS) of nuclear ribosomal DNA except 5.8S have shown broadly utility across land plants (with the exception of ferns) and has been considered as potential barcodes locus [9,10]. Second, molecular systematic investigations at introns of the chloroplast and ITS. For instance, Kress et al. [9] compared several loci of tobacco and deadly nightshade in 7 plant families and a total of 99 species belonging 88 genera in 53 families, and they purposed that the psbA-trnH spacer and ITS could be used as the most plastid region in angiosperms with easily PCR amplification across wide stretches range of land plants. However, ease of amplify and sequence generation with universal primer pairs and reaction conditions was determined this pair failed to provided particular identifiers for the whole members of Cycadales [11]. Analysis over 1000 rbcL sequences from GenBank found that rbcL could authenticate samples in approximately 85% of pairwise comparisons of congeneric species [12]. In other study, the discriminatory increased to about 88% when a combination of a non-coding trnH-psbA spacer and plastid coding genes rbcL was used across a set of 48 genera from 43 families, totally 96 diverse species [13]. Lahaye et al. [14] analyzed >1000 of Mesoamerican orchids (295 species two representatives) and identified that a section of plastid gene could be a putative barcode loci for flowing plants. Altogether, a number of markers of the plastid genome mainly in four coding (rpoB, rpoC1, matK, rbcL) and three non-coding (trnH-psbA,psbK-psbI and atpF-atpH) regions have shown high qualities as DNA barcodes in land plants [9,14,15,16,17]. Recently, overwhelming landmark articles on DNA barcoding have been published (**Table 1**) on account of high conserved sites to provide new methods of Chinese medicinal material identification.

Table1. Some applications of DNA barcoding published in the identification of herbal materials onward 2003.

Year	Article	References
2003	Phylogeny of Astragalus in China: molecular evidence from the DNA sequences of 5S rRNA spacer, ITS, and 18S rRNA	Dong et al. [18]
2005	Phylogenetic relationship of Glycyrrhiza lepidota, American licorice, in genus Glycyrrhiza based on rbcL sequences and chemical constituents	Hayashi et al.[19]
2006	Differentiation of Dendrobium species used as “Huangcao Shihu” by rDNA ITS sequence analysis	Xu et al. [20]
	Identification of medicinal Atractylodes based on ITS sequences of nrDNA	Shiba et al. [21]
	Sequence analysis of chloroplast chlB gene of medicinal Ephedra species and its application to authentication of Ephedra Herb	Guo et al. [22]
2007	Molecular authentication of Radix puerariae lobatae and Radix puerariae thomsonii by ITS and 5S rRNA spacer sequencing	Sun et al.[23]
	ITS sequence analysis used for molecular identification of the Bupleurum species from northwestern China	Yang et al. [24]
	Molecular analysis of the genus Mitragyna existing in Thailand based on rDNA ITS sequences and its application to identify a narcotic species: Mitragyna speciosa	Sukrong et al. [25]
	Identification of Dryopteris crassirhizoma and the adulterant species based on cpDNA rbcL and translated amino acid sequences	Zhao et al. [26]
2008	Authentication of Saussurea lappa, an endangered medicinal material, by ITS DNA and 5S rRNA sequencing	Chen et al. [27]
	Sequencing analysis of the medicinal plant Stemona tuberosa and five related species existing in Thailand based on trnH-psbA chloroplast DNA	Vongsak et al. [28]
2009	Identification of crude drugs from Chinese medicinal plants of the genus Bupleurum using ribosomal DNA ITS sequences	Xie et al. [29]
	Molecular identification of Hypericum perforatum by PCR amplification of the ITS and 5.8S rDNA region	Howard et al. [30]
	Identification of Verbena officinalis based on ITS sequence analysis and RAPD-derived molecular markers	Ruzicka et al. [31]

2010	Molecular authentication of the Chinese herb Huajuhong and related medicinal material by DNA sequencing and ISSR marker	Su et al. [32]
	Molecular analyses of the Chinese herb Leigongteng (<i>Tripterygium wilfordii</i> Hook.f.)	Law et al. [33]
	Detection of <i>Valeriana jatamansi</i> as an adulterant of medicinal Paris by length variation of chloroplast psbA-trnH region	Yang et al. [34]
	Cardiocrinum seeds as a replacement for <i>Aristolochia</i> fruits in treating cough	Li et al. [35]
	Molecular authentication of the ethnomedicinal plant <i>Sabia parviflora</i> and its adulterants by DNA barcoding technique	Sui et al. [36]
	Identification of medicinal <i>Dendrobium</i> species by phylogenetic analyses using matK and rbcL sequences	Asahine et al. [37]
	Evaluating the feasibility of using candidate DNA barcodes in discriminating species of the large Asteraceae family	Gao et al. [38]
	Identification of medicinal plants in the family Fabaceae using a potential DNA barcode ITS2	Gao et al. [39]
	Authentication of <i>Taxillus chinensis</i> using DNA barcoding technique	Li et al. [40]
	Species Identification of Medicinal Pteridophytes by a DNA Barcode Marker, the Chloroplast psbA-trnH Intergenic Region	Ma et al. [41]
2011	Using DNA Barcoding to Identify Species within Euphorbiaceae	Pang et al. [42]
	DNA barcoding of the Lemnaceae, a family of aquatic monocots	Wang et al. [43]
	Identification of Fabaceae Plants Using the DNA Barcode matK	Gao et al. [44]
	Testing the potential of proposed DNA barcodes for species identification of Zingiberaceae	Shi et al. [45]
	Identification of <i>Lonicera japonica</i> and its Related Species Using the DNA Barcoding Method	Sun et al. [46]
2012	Identification of herbal medicinal materials using DNA barcodes	Li et al. [47]
	Applying plant DNA barcodes for Rosaceae species identification	Pang et al. [48]
2013	Genome sequence of the model medicinal mushroom <i>Ganoderma lucidum</i>	Chen et al. [49]
	Comparison of four DNA barcodes in identifying certain medicinal plants of Lamiaceae	Han et al. [50]
2014	Use of the potential DNA barcode ITS2 to identify herbal materials	Pang et al. [51]
	A fast SNP identification and analysis of intraspecific variation in the medicinal <i>Panax</i> species based on DNA barcoding	Chen et al. [52]
	Application of the ITS2 Region for Barcoding Medicinal Plants of Selaginellaceae in Pteridophyta	Gu et al. [53]
2015	Identification of cortex herbs using the DNA barcode nrITS2	Sun et al. [54]
	DNA barcoding of medicinal plant material for identification	Techen et al. [55]
	Identification of species in the angiosperm family Apiaceae using DNA barcodes	Liu et al. [56]
	A renaissance in herbal medicine identification: From morphology to DNA	Chen et al. [57]
	DNA barcoding Chinese medicinal <i>Bupleurum</i>	Chao et al. [58]
	Evaluation of the DNA Barcodes in <i>Dendrobium</i> (Orchidaceae) from Mainland Asia	Xu et al. [59]

Herbal medicinal materials can be supplanted accidentally or intentionally by materials from closely related species or unrelated plants [60,61]. Adulteration of herbal materials often occurs because of (i) the materials do not have clearly diacritical morphological characters (ii) the materials having similar names (iii) the materials is expensive and valuable herbs, but the substitution is economically. Government officials and the pharmaceutical industry have the responsibility to guarantee that any medicinal materials are genuine, and that they are safe for consumers. And, it becomes difficult for the specialists to diagnostic species correctly. Therefore, using a standard DNA barcodes can offer a practical solution to discriminate herbal medicinal materials and their adulterants.

DNA barcodes for identifying herbal medicinal materials

Adulterants of herbal materials approximately derived from closely related species or plant parts that obtain similar morphological. Herein, DNA barcodes online databases that can identify Chinese herbal materials at different taxonomic by various reference sequence. Several DNA regions such as ITS, ITS2, psbA-trnH, rbcL, matK has been suggested as important potential candidates for plant DNA barcodes. The common primers used for the amplification and sequencing of the barcoding regions are given in **Table2**.

Table2. Universal primers for PCR amplification and DNA sequencing.

Gene	Name of primer	Primer sequence	Reference
ITS	5a fwd	5'CCTTATCATTAGAGGAAGGAG-3'	Kress et al.[9]
	4 rev	5'-TCCTCCGCTTATTGATATGC-3'	
ITS2	S2F	5'-ATGCGATACTTGGTGTAAT-3'	Chen et al. [62]
	S3R	5'-GACGCTTCTCCAGACTACAAT-3'	
psbA-trnH	fwd PA	5'-GTTATGCATGAACGTAATGCTC-3'	Sass et al. [11]
	rev TH	5'-CGCGCATGGTGGATTACAAATCC-3'	
matK	390F	5'-CGATCTATTCATTCAATATTC-3'	Cuenoud et al. [63]
	1326R	5'-TCTAGCACACGAAAGTCGAAGT-3'	
rbcL	1F	5'-ATGTCACCACAAACAGAAAC-3'	Olmstead et al.[64]
	724R	5'-TCGCATGTACCTGCAGTAGC-3'	

CBOL Plant Working Group compared the performance of seven candidate plastid DNA regions (matK gene, rbcL gene, rpoB gene, rpoC1 gene, trnH-psbA spacer, psbK-psbI spacer, and atpF-atpH spacer) in 907 samples representing 38 gymnosperm, 67 cryptogam, and 445 angiosperm species and recommended the combination of rbcL+matK as the standard plant markers^[66]. The Barcode of Life Data System (BOLD) was established by Canadian Centre and has constructed the first online DNA barcoding database (<http://www.boldsystems.org>). This database can store, analyse and publish DNA barcodes, which is made freely publically available by assembling molecular and it bridges a renaissance in Chinese crude drugs identification^[67]. Medicinal Materials DNA Barcode Database has totally 18436 sequences available in 1259 species, which contains basic information, all plastid DNA regions and the internal transcribed spacer of nuclear ribosomal cistron for the medicinal materials^[68]. An elementary system for identifying Chinese herbal materials has been established based on the two loci of ITS2+psbA-trnH including 78847 sequences from 23262 species^[69]. The system concluded ITS2 is the core standard plant barcode and the usefulness psbA-trnH region can be regarded as a supplementary barcode in medicinal plants. Here, we summarize key research advancements in the DNA barcoding of herbal materia medica.

Nuclear ribosomal internal transcribed spacer (ITS) region

The internal transcribed spacer (ITS) region of the nuclear ribosomal cistron is a multigene sequence encoding the nucleic acid of the ribosome. Within the cell, the cistron is consisted of three repeated and conserved units coding regions (18S, 5.8S, 26S) and two internal transcribed spacers (ITS1 and ITS2). This region based on the biparentally inherited and hybridization events is equipped to provide more genetic information on species identify and has been tested for calculated as plant barcodes^[9,11,70]. ITS has high species discrimination and PCR amplification, so the cistron is considered as the most frequently sequenced region in plant phylogenetic studies. Similarly, it is the most regular barcoding region used for herbal medicinal materials authentication. For example, Xue & Li^[71] provides the ITS could successfully distinguish traditional Tibetan medicinal plant (*Gentianopsis paludosa*) including their adulterants. The ITS rDNA sequencing was applied successfully to differentiate *H. diffusa* from *H. corymbosa*, a Chinese herb for cancer treatment, and other adulterants of Baihuasheshcao^[72]. Although, ITS has the high variation and species discrimination in some genus, the interspecific percentages of the ITS region is low in some instances. For instance, Overall, ITS is variable too far including insertions and deletions and it cannot ensure reliable alignments.

Universal primers are an important touchstone for an ideal DNA barcoding. The great problems with ITS is the lack of universal primers hindered PCR conditions and sequencing success. The ITS sequence contains sufficient variable sites for species identification, but ITS is too long to extract intact DNA from some samples due to the highly degraded DNA that happened in the museum specimens and herbs from the market during long storage period. Overcoming the limitation of entire ITS, ITS2 is a much shorter and better sequence for herbal material discrimination because of its more conserved sequence, which reduces erroneous amplification. The high variability and species-distinguishment of the ITS2 intergenic spacer apply as a core DNA barcode for Chinese materials. This candidate barcode possessed high interspecific divergence and low intraspecific genetic variations, which successfully identified 92.7% at the species level from more than 6600 plant samples of 4800species in 753 genera^[62]. The usefulness of ITS2 for Chinese medicines has been tested in many studies. For instance, in Asteraceae species, five DNA regions (rbcL, matK, ITS, ITS2 and trnH-psbA) was valuated using large plant taxon, including various medicinal plants and related species. The results reveal that ITS2 correctly authenticated 76.4% at the species level but at the general levels is 97.4% derived from 2315 species pertaining to 494 genera^[38]. Gao et al.^[39] shows that the ITS2 sequence can be used to identify twenty-four Fabaceae species in the Chinese Pharmacopoeia including another 66 species and their related species. The data present that the ITS2 region can not only correctly distinguish over 80.0% of species but also successfully differentiated 100% of genera in the 1507 sequences of 1126 Fabaceae species^[39]. In the recent years, Shi et al.^[45] compared the identification efficiency of ITS2, rbcL and other universal barcode, and the results suggest that the ITS2 has the highest interspecific variation and “barcoding gap” between interspecific variation and intraspecific divergence to discriminate Zingiberaceae species. Subsequently, Chen et al.^[52] applied single nucleotide polymorphisms (SNPs) to detect the intraspecific divergence in ITS2 of medicinal *Panax* species, and the multi-copy ITS2 could be deemed as an ideal locus in the genomes of *Panax ginseng* and *Panax quinquefolius* two species. The ITS2 barcode also performed good exhibition at identifying Rosaceae^[48], Rutaceae^[73], Lamiaceae^[50], Euphorbiaceae^[42]. These examples indicate that the ITS2 barcode is a forceful tool with which land plants and herbal materials can be distinguished.

Although the DNA barcode can assist to discriminate herbal medicinal materials with ITS region and ITS2 intergenic spacer. However, the two regions sometimes may lead to a mass of erroneous identification. Likewise, the existence of multiple copies and the secondary structure can result in poor quality PCR amplification due to the hybridization and biparental inheritance of ITS2 region^[74]. Endophytic fungi contamination is usual in herbal materials, which may lead to improperly PCR results. Because fungi and herbs both include ITS2 sequence, we could simultaneously examine the gene sequences. To overcome this issue, effective experimental methods including specific primers and conditions should be adopted to avert fungal contaminants^[75].

trnH-psbA intergenic sequence

The trnH-psbA intergenic spacer is one of the most mutative sequences in the chloroplast genome. This intergenic sequence has an average length of approximately 450 bp, but varying from 247 to 1120 bp^[66,70]. This intergenic spacer shows the highest species discriminatory power and amplification success rate among nine putative locus tested across a broad range of taxa (include angiosperms, gymnosperms, ferns, mosses, and liverworts)^[9,13]. Recent studies have demonstrated the trnH-psbA region

can be used as a useful DNA fragment for the discrimination of medicinal plants and their adulterants. Herba Dendrobii is one of the most useful groups in traditional Chinese materials, Yao et al. [76] have been sequenced the chloroplast psbA-trnH intergenic spacer to distinguish the 17 medicinal species Dendrobium and one Bulbophyllum odoratissimum with the average percentages of nucleotide 2.5% (range from 2.0% to 3.1%). It also exhibits high interspecies percentages of nucleotide (ranging from 0.3% to 2.3%) and low intraspecies percentages of nucleotide (ranging from 0% to 0.1%) among all Dendrobium species. *Lonicera japonica*. Thumb (Caprifoliaceae) is widely known in Chinese Pharmacopoeia for its detoxifying, and anti-inflammatory functions. Sun et al. [46] tested seven candidate DNA barcodes on forty-four samples of *Lonicera japonica* and their closely related species, using the six parameters and Wilcoxon signed rank tests. The non-coding psbA-trnH DNA barcode yield 100% species identification efficiency and the highest interspecific divergence. In the recent study, Song et al. [77] employed eight DNA barcodes to authenticate thirty-eight specimens belonging to eighteen species of the family Polygonaceae in Chinese pharmacopoeia. As the result, the chloroplast psbA-trnH can not only identify eighteen species of Polygonaceae, but also distinguish eight other species including some commonly used adulterants of Polygonaceae out of Chinese pharmacopoeia. As for medicinal pteridophytes, Ma et al. [41] detected five DNA markers of six plastid genomic sequences from GenBank and found the psbA-trnH intergenic sequence has available universal primers of 51 pteridophyte species from 24 families. Next, they showed the psbA-trnH intergenic sequence has both high PCR amplification efficiency of 94.1% and high sequencing success rate of 81.3%. Although, the psbA-trnH is useful to identify many Chinese herbal species, it was difficult to differentiate and sequence *Glyceria* and *Heracleum* [78,79].

One disadvantage of the psbA-trnH region is that the sequence is too long in some species (>1000 bp) to generate bidirectional unambiguous regions. Because of its length, the use of this region as herbal DNA barcode alone is problematic to extract high quality DNA from degraded materials or forensic samples [70]. The other weakness is the presence of a poly-A/T structure in the psbA-trnH region, which caused problematical in sequence alignment. In addition, the number of nucleotide insertions and deletions is more frequent than in other positions [80]. The rps19, rpl22 genes and pseudogene is also found in the middle of psbA-trnH in many species, such as Zingiberales, Dioscoreales and Liliales [81]. Overall, some possible bioinformatics solutions have been implemented to deal with the enormous length issue and molecular evolution.

MaturaseK gene (MatK)

The highly conserved matK of chloroplast gene is about 1500 bp long, which is located in the intron of the trnK. And, this region encodes a maturase-like protein within the Group II intron splicing. The gene exhibits a high rate of substitution and high levels of discrimination among species. Thus, the matK gene can be emerging as a potential candidate for the study of plant systematics and evolution [66]. Among angiosperm species, the matK gene has been adopted as a momentous barcode by a large amount of researchers [14,82,83]. In plant species, the Consortium for the Barcode of Life (CBOL) Plant Working Group proposed the conjunction of rbcL+matK represents a pragmatic solution to molecular identification of plants [66]. Based on the high discrimination of the matK region, this gene is commonly used to distinguish Chinese herb materials of different geographical population, especially sister species. In addition, the traditional Chinese materials Zingiberaceae family members could be identified from species discrimination power and phylogenetic relationships by exercising the chloroplast matK gene [83]. The sequence comparison of matK gene within the Fabaceae family revealed that the sequence had high applicability as the core DNA barcode with approximately 29 genera in over 53 species [44]. As computed by six parameters and BLAST method [84,62], the matK gene confirmed a greater level between species and genetic divergence within species, that yield high discrimination power at species and genus [44]. Nonetheless, the most crucial challenge for plant barcoding is the capacity to identify sister species across large range of geographical populations. Although some chloroplast sequences have significant ability to identify invasive species, a small number of sequences with low plastid variation failed in certain taxonomic groups.

However, the shortcoming of the matK region as a universal barcode is the absence of versatile primers [70, 85,86]. In order to circumvent the problem, we present that matK be used bond with other universal markers as DNA barcodes. In addition, new sets of primers need to be developed in the region. Regarding the high evolutionary rate of matK, it has been exhibited either alone or incorporation with other locus.

rbcL coding gene

Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) is a bifunctional enzyme, which can concentrate the reaction for CO₂ and ribulose-1,5-bisphosphate (RuBP) to generate two molecular 3-phosphoglyceric acid (the first step of CO₂ fixation). Moreover, it has the ability to assimilate O₂ to disintegrate RuBP. Rubisco comprised of eight large subunits that is encoded by plastid rbcL gene and eight small subunits. The eight large subunits mainly include photosynthetic sites of the enzyme. The merits of the rbcL gene are that it has universal primers and ease of amplification and alignment [11,13,14]. We pay attention to rbcL gene because the coding region has a large of rbcL sequences in Genbank, with wide survey from major groups, and will provide the rbcL sequence for a first tier barcoding of most land plants [12]. Recently, using the rbcL region in conjunction with one or more than one DNA markers results in higher identification rate and discrimination power of species than the single locus [13]. Subsequently, the Consortium for the Barcode of Life (CBOL) Plant Working Group (PWG) recommended the combination of rbcL+matK as core barcodes and ITS/ITS2 as the complementary barcode for distinguishing plants at the Third International Barcoding Conference [66]. Therefore, the chloroplast rbcL gene is one of the best putative barcode in the phylogenetic and systematics analyses of land plants. Many experimental investigations were also observed in herbal medicinal materials and their related adulterants. For

example, Gao et al. [38] evaluated five regions for discriminating species in the Asteraceae family. The results showed that *rbcL* gene has high amplification and sequencing efficiency, but the region exhibited the lowest inter-specific divergence to distinguish species. Low interspecific variation was also found in the experimental consequence of Euphorbiaceae, Rosaceae, Zingiberaceae and Polygonaceae [42,48,83,77]. The demerit of the *rbcL* sequence is that the significantly length of the gene is too long (at least 1300 bp long) to cause problems. Usually, it is significant to make use of four primers for bidirectional sequencing of the entire gene [9].

DISCUSSION AND CONCLUSIONS

The identification of Chinese medicinal materials is a global concern for ensuring safety reasons and therapeutic effectiveness to raise consumers' confidence. Traditional methods of authentication are loose and unable to reach the need for the rapid and automatable identification for herb materials. Therefore, DNA barcoding surmount the issue and with the purpose of generate the universal standard. However, each molecular marker has its own benefits and drawbacks; there is no standard primers and universal DNA barcode to authenticate different herb material. For future research, it is necessary to ameliorate the DNA barcoding system of Chinese herb materials.

Successful genomic DNA is an essential prerequisite for authenticating herb materials using DNA barcoding techniques. As for fresh materials, the methods of DNA extraction are relatively easy. But the majority of traditional Chinese herb materials are regular procured from the dried or powdered plants that were collected from the markets, including roots, stems, leaves and flowers. Moreover, the physiological conditions, harvest seasons, environmental factors, storage and processing methods are different for each herb. Therefore, DNA extraction of those materials should be handled carefully to avert DNA degradation and fungal contaminants. In most cases, Chinese medicinal materials are usually plant parts that contain high levels of phenolic compounds, polysaccharides and pigments. The issues with contamination of endophytic fungi widespread exist, which may be valuable inhibitors to PCR amplification. In practice, effective experimental methods could help to reduce the probability of undesirable consequences. Generally, using 75% alcohol to clear the surface of herbal material preceding DNA extraction can prevent endophytic fungi DNA extraction and ostensible impurity.

In conclusion, the DNA barcode is not only effective in identifying herbal materials, but it is also a resultful tool for distinguishing closely species or substitute species among herbal materials. We believe that the development of a reference DNA barcode system for Chinese medicinal materials will assist in improving the safety of drug use in the context of increasing potential adverse reaction.

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