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HOW TO CORRECTLY IDENTIFY HERBAL MATERIALS IN MARKET: A CASE STUDY BASED ON DNA BARCODES

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Abstract

Background: Traditional methods for identifying herbal medicines have many shortcomings. In this study, we aim to test discriminating ability of DNA barcodes and explore feasible method on evaluating identification results.
Materials and Methods: Materials of whole-plant medicines were sampled from herbal market. 111 samples were used for DNA sequencing of ITS2 and *trnH-psbA* regions. Assembled sequences were searched against reference database using the BLAST method. Comprehensive evaluation based on pharmacognostic investigation, macroscopic identification and identification of DNA barcodes were performed for authentication of the herbal materials.
Results: As a result, ITS2 had better identifying power than *trnH-psbA* in species-specific level (55.86% & 45.95%), as well as worse success rate of DNA sequencing (74.58% & 94.59%). In total, 89.19% individuals could be identified in genus level at least.
Conclusion: It was revealed that DNA barcoding was useful tool in identifying herbal materials. Both ITS2 and *trnH-psbA* should be incorporated into the standard regions of DNA barcodes for identifying herbal materials.

Keywords: DNA barcodes, herbal materials, ITS2, *trnH-psbA*, identification

Introduction

Traditional medicines are widely used to cure diseases and maintain health in the long history throughout many cultures and regions (Chen et al., 2010; Li et al., 2011b; Pang et al., 2013). Over the past decade, there has been a dramatic increase in the demand for these medicines and pharmaceutical products in both developing and developed countries (Heubl, 2010; Lee et al., 2008). Traditional medicines are extremely rich and diverse in China, including traditional Chinese medicine (TCM) and various ethnomedicines. They account for about 40% of all health care (Lee et al., 2008). However, a severe problem on medicinal market is that many erroneous substitutes and adulterants are traded due to their lower costs or misidentification of species with similar morphological features (Gutteridge and Burns, 2013; Heubl, 2013). There were several cases that the adulterants or substitutes caused serious intoxication and even deaths (Chan and Critchley, 1996; Ernst, 2002; Heubl, 2010; Zhao et al., 2006). Authentication of these traditional medicines and their adulterants is an essential prerequisite to ensure safety, herbal drug quality and therapeutic efficacy (Heubl, 2013). In practice, identification of herbal materials mainly relies on morphological (macroscopic and microscopic) and chemical analyses. But these methods often have obvious weakness (Heubl, 2010).

DNA barcoding, a new approach for species identification using a short, standardized DNA region, has become an important tool in distinguishing species, discovering cryptic species, protecting endangered species and identifying traditional medicine etc. (Chen et al., 2010; Hebert et al., 2003; Kress et al., 2005; Li et al., 2011a; Techen et al., 2014). In animal barcode, the mitochondrial gene cytochrome c oxidase subunit 1 (*CO1*) shows extremely great efficiency. However, the barcode is faced with serious challenge in plant due to its low substitution rates (Hollingsworth et al., 2011). Many botanists have done a lot of researches on selecting appropriate regions and their combinations (Chase et al., 2007; Chen et al., 2010; Hollingsworth et al., 2009, 2011; Kress et al., 2005, 2007; Li et al., 2011a). A two-marker combination of plastid *rbcL* and *matK* was recommended as the core plant barcode, to be supplemented with additional markers such as plastid *trnH-psbA* and nuclear ribosomal internal transcribed spacer (ITS) (Hollingsworth et al., 2009). However, Chinese experts proposed that ITS/ITS2 should be incorporated into the core barcode for seed plants (Chen et al., 2010; Li et al., 2011a; Yao et al., 2010). For herbal materials, it is more difficult to identify because their genome DNA is often seriously degraded in harvesting and prepared progress. So DNA barcodes for herbs should be easy to be amplified and sequenced. It was proposed that ITS2 served as a universal barcode for identifying plant medicine, plus *trn*H-*psb*A as a complementary barcode (Chen et al., 2010; Han et al., 2013; Pang et al., 2013; Sun and Chen, 2013; Yao et al., 2010). Meanwhile, establishment of open-access molecular databases has laid foundation for DNA barcoding to impart efficient identification of herbal medicine (Gutteridge and Burns, 2013).

The Third Month Fair is a renowned traditional commodity fair in Dali prefecture, Yunnan province (Liu, 2012). This fair is a grand gathering for tourism, trade, culture and sport, possessing important impact on Dali and adjacent regions. Trade of traditional medicines is an important part of this fair. Besides professional druggists, local herbalists are also important partner who usually sell herbal medicines with local characteristics (Zhang et al., 2014). Whole herb is a popular type of plant medicines. It is lack of distinguishing morphological characters generally. So this herbal material is more difficult to be identified. In the present study, we aim to test identified ability of the DNA barcodes in identifying herbal materials in market and explore feasible method on evaluating identification**.**

Materials and Methods

Medicinal materials and pharmacognostic investigation

During the Third Month Fair in 2013, 116 samples of whole herb were collected from local herbalists in medicinal market (Fig.1). For each sample, about 100g herbal materials without contamination was prepared and put into valve bag as voucher specimens and molecular materials.

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Meanwhile, pharmacognostic investigation was performed to obtain relative information of the sampled materials, including local name, producing area, collecting time and medicinal value. All the specimens were deposited in the Herbarium of Medicinal Plants and Crude Drugs of the College of Pharmacy and Chemistry, Dali University.

Figure 1: Herbal market in the Third Month Fair and part herbal materials in the present study.
(a. local people in herbal market; b. local herbalists and his herbal materials; c. Luxiancao (QC013), *Pyrola decorata*; d. Guizhencao (QC055), *Bidens pilosa*; e. Xixiancao (QC064), *Siegesbeckia orientalis*; f. Cheqiancao (QC005), *Plantaog major*.

Macroscopic identification

Each medicinal material was firstly identified by macroscopic characters on basis of pharmacognostic investigation. The identification was progressed according to relative reference books, mainly including the *China Pharmacopoeia* (Chinese Pharmacopoeia Committee, 2010), the *Drug Standard of Yunnan Province* (Yang, 1996) and the *Atlas of Chinese Herbal Medicine and Ethnomedicine* (Huang, 2005) etc. Putative scientific name of original plant was determined based on the aforementioned books and rechecked using the *Flora of China* (Wu and Peter, 2012).

Genomic DNA extraction, amplification and sequencing

Total DNA was extracted from clean herbal materials using modified CTAB method (Doyle and Doyle, 1987). Universal primers for ITS2 and *trn*H-*psb*A, as well as their corresponding reaction systems and procedures were obtained from previous studies (Chen et al., 2010; Li et al., 2011a; Yao et al., 2010). Purified PCR products were sequenced in both directions with the primers used for PCR amplification on the ABI 3730XL sequencer (Applied Biosystems, USA). Only DNA sequences in accordance with corresponding standard were used for final analysis (Chen et al., 2010; Li et al., 2011a).

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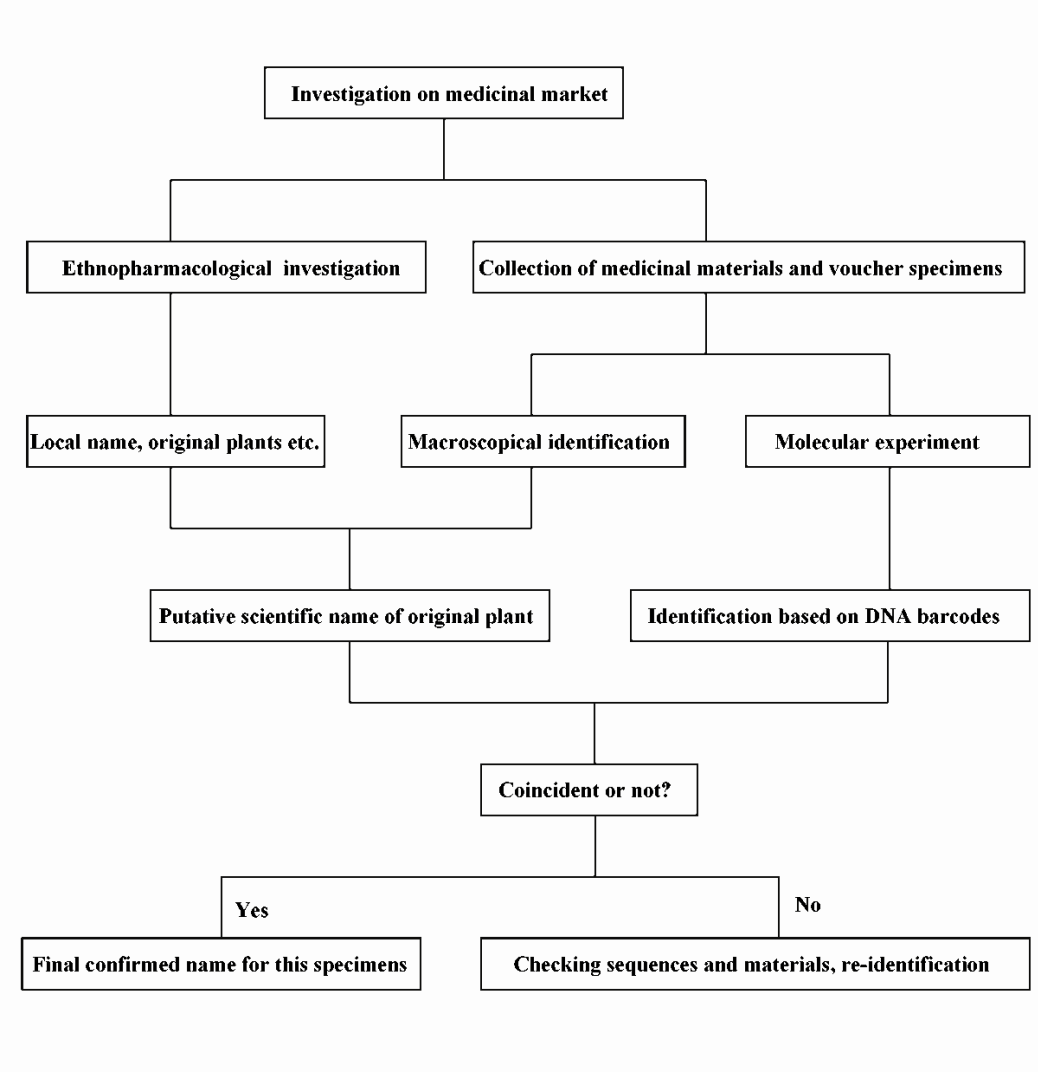

Figure 2: Graph for identifying herbal materials in market though DNA barcodes.

Sequencing alignment and molecular identification

Bidirectional sequences were assembled using the program SeqMan Pro 7.1.0 (DNASTAR, Lasergene). They were not aligned due to enormous variation among DNA sequences from different samples. BLAST (basic local alignment search tool), as the most successful way to identify the market samples, was performed for all individuals in the present study (de Boer et al., 2014; Kool et al., 2012). The sequence for each material as a query sequence was submitted and blasted firstly in the DNA Barcoding System for Identifying Herbal Medicine (http://www.tcmbarcode.cn/en/) (Until April 23, 2014). The species with the nearest match, namely highest similarity was the closest species to species of the query sequence (Chen et al., 2013). In the present study, this species was regarded as possible identified species of original plant for the herbal material though DNA barcodes. But the identified result would be denied if several species were extremely close to query sequence. Meanwhile, result of molecular identification should be coincident to putative species according to macroscopic identification and pharmacognostic investigation, or else the sample would be re-identified or treated as failure identification. The samples with unconfirmed or failure identification above would be further blasted and identified in the National Center for Biotechnology Information (NCBI, http://www.ncbi.nlm.nih.gov/) (Boratyn et al., 2013) (Until April 23, 2014). NCBI's web-based megablast algorithm using the default settings was used to identify the query sequences as proposed (Chen et al., 2013; Kool et al., 2012). Final identification was made manually, taking E-value, maximum identity, number of closely related species represented in the database, as well as distribution of the plants in question into consideration(de Boer et al., 2014). Confirmed scientific name of original plant for identified sample was determined based on the two DNA barcodes and macroscopic identification (Fig.2).

Results

Macroscopic identification and pharmacognositc investigation

In this study, 116 samples were collected from the medicinal market. Among these materials, 111 samples were used for molecular experiment and final analysis except 5 with contaminants or rot. All herbal materials had local names according to pharmacognostic investigation on herbalists. A few of names with mistake in investigation were revised. On the basis of relative reference books, putative scientific name of original plant were determined. Among the 111 samples, original plants of 88 and 15 individuals could be determined in species and genus lever, as well as 8 were

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unconfirmed (Table 2).

DNA extraction, PCR amplification and DNA sequencing

Total DNA was extracted out successfully for all samples. For DNA barcodes, success rates of PCR amplification were 98.20% for *trnH-psbA* and 90.99% for ITS2. Dual-band in amplified product was detected in ITS2 region. Only good amplified products were used for further DNA sequencing except that with failure amplification and dual-band. Moreover, success rates of DNA sequencing were relatively low for ITS2 (84.16%) but it was much better for *trnH-psbA* (96.33%). In final, 85 samples for ITS2 (76.58%) and 105 samples for *trnH-psbA* (94.59%) obtained usable DNA sequences that were used for identification though DNA barcodes (Table 1).

Table 1: Statistical data for molecular experiment of DNA barcodes.

Molecular experiment	Experiment result	ITS2	*trnH-psbA*
PCR amplification		111	111
	Success	101	109
	Dual-band	2	No
	Failure	8	2
	Success rate	90.99%	98.20%
DNA sequencing		101	109
	Success	85	105
	Overlap pink	4	3
	Failure	14	1
	Success rate	84.16%	96.33%
Final success rate		76.58%	94.59%

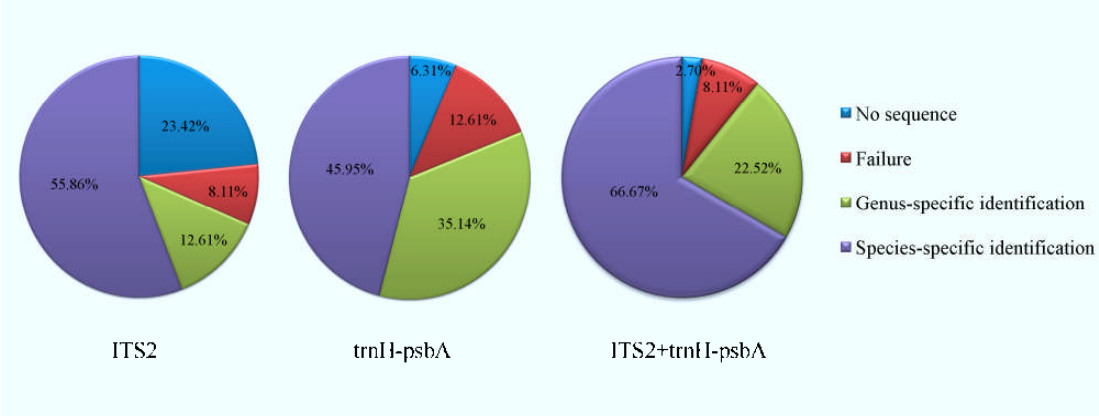

Figure 3: Identified results of ITS2, *trnH-psbA* and their combination.

Identification though DNA barcodes

For ITS2 region, 55.86% and 12.61% samples could be identified in species-specific and genus-specific level. Meanwhile, there were 23.42% individuals without usable sequences and 8.11% could not be correctly identified by ITS2 region. For *trnH-psbA*, species-specific identification was obviously lower than ITS2 (45.95%) but genus-specific identification was relatively high (35.14%). However, individuals without usable sequences for *trnH-psbA* region (6.31%) were much fewer than ITS2 (23.42%). On the basis of coalition analysis of the two DNA barcodes, 98 samples (89.19%) could be identified in genus level at least. All the statistical data were shown in Fig. 3 and Table 2.

Among the 111 samples, original plants of 8 were unconfirmed according to macroscopic identification and pharmacognostic investigation. Most of them could be correctly identified in species-specific (4/8) or genus-specific (2/8) level. Meanwhile, identification based on DNA barcodes successfully revealed adulterants (14/111) in sampled herbal materials. Furthermore, 12 substitutes were also found by DNA barcodes in this study.

There were 98 samples that were successfully identified at genus or species level. Their original plants were relatively rich in Asteraceae (18) and Lamiaceae (14). Some herbal materials were from original plant that had massive relative species. They were extremely difficult to be correctly identified regardless of macroscopic or molecular identification, such as *Adiantum*, *Artemisia* etc.

Discussion

Trade of herbal materials is an important part of the Third Month Fair. There were 362 plant medicines at least that were correctly identified and recorded according to incomplete statistics (Zhang et al., 2014). However, massive substitutes and adulterants of genuine herbal materials also arise in herbal market. Whole herb is often difficult to be identified using macroscopic identification because they are usually badly wrinkled or fragmentized. Microscopical and physic-chemical identifications are popular methods as well, but they need relatively high professional quality for authenticator and their discriminatory ability is limited. On the contrary, identification though DNA barcodes, possessing obvious merits compared with the above

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methods becomes a better choice to identify herbal materials (Heubl, 2010). DNA barcoding opens up a unique avenue for the identification of organisms (Li et al., 2011a). It provides a powerful tool to complement chemical analyses for authentication of Chinese medicinal plants and to ensure that herbal materials are not contaminated with ineffective or potentially harmful substitutes or adulterants (Buriani et al., 2012; Heubl, 2010). Herbal materials are generally difficult for molecular identification because their genome DNA usually degraded seriously due to processing treatment (Coghlan et al., 2012). So DNA barcodes for identifying herbal materials should have great usability and variation. Previous data suggested that ITS2 represented the most suitable region for DNA barcoding applications so it was proposed as a novel universal barcode for herbal materials (Chen et al., 2010, 2013; Yao et al., 2010). Until now, the barcode has showed excellent identified power on medicinal materials (Pang et al., 2013; Sun and Chen, 2013). Meanwhile, *trnH-psbA* region, possessing great usability and reliability for species authentication, is also listed as a complementary barcode to ITS2 (Chen et al., 2010, 2013).

In this study, both ITS2 and *trnH-psbA* regions were used to identify herbal materials but they showed obviously difference in usability and identifying ability. Success rates of PCR amplification and DNA sequencing for ITS2 were extremely lower than that for *trnH-psbA* region (Table 1). Only 85 samples got usable DNA sequences for ITS (76.58%); meanwhile, 105 samples were successfully sequenced for *trnH-pasbA* (94.59%). Usability of ITS2 here was not such ideal as proposed (Chen et al., 2010; Pang et al., 2013; Yao et al., 2010). But these studies were performed using medicinal plants in which better DNA quality was deposited. There were a few similar studies for identifying herbal materials using the BLAST method of the two barcodes (Kool et al., 2012; Sun and Chen, 2013). So no suitable referenced data could be used here. On the contrary, ITS2 region showed better discriminatory ability than *trnH-psbA* in species-specific identification (55.86% & 45.95%); whereas, they were converse in genus-specific level (12.61% & 35.14%). It revealed that ITS2 possessed better discriminatory ability than *trnH-psbA* region without consideration of its low success rate of DNA sequencing. The two regions possessed obvious advantage and disadvantage each other. In total, 89.19% of all the herbal materials could be correctly identified in genus-specific level at least. It showed that combination of the two DNA barcodes could obviously improve identifying power compared to any single barcode. So we propose that both ITS2 and *trnH-psbA* should be listed as standard regions of DNA barcodes for herbal materials.

In this study, DNA barcodes also showed excellent power in identifying counterfeits and adulterants of certified reference materials. There were 14 counterfeits and 12 adulterants which were correctly identified from sampled materials. For example, “Baihuasheshecao” (QC002) was a common local medicine possessing anti-inflammatory, anti-tumor and antiviral values (Huang, 2005). Its original plant is *Hedyotis diffusa*, but the sample was identified to be counterfeit, namely *Arenaria serpyllifolia*. Moreover, some unknown herbal materials were correctly identified on basis of the DNA barcodes. Original plant of “Maticao” (QC024) was not confirmed according to macroscopic identification and pharmacognostic investigation which was successfully identified by DNA barcodes, namely *Centella asiatica*. So the method is reliable tool for identifying herbal materials, their substitutes and adulterants, as well as unknown medicines (Techen et al., 2014).

Although DNA barcode is beneficial to identify herbal materials it still needs assistance from traditional methods, including herbal information from herbalists and macroscopic identification etc. In fact, two aspects of studies are equally important. On the one hand, pharmacognostic investigation or communication with druggists can afford rich information for herbal materials. But the information may be incorrect. So further macroscopic identification using reference book, such as the *Chinese Pharmacopoeia* is necessary that also provides formal information. On the other hand, identification though DNA barcodes give confirmed result or clue. Basic method of identification though DNA barcodes is BLAST searches for query sequences in open-access database, such as the DNA Barcoding System for Identifying Herbal Medicines (Chen et al., 2013). This is a specific platform for identifying traditional medicines by DNA barcodes regions which affords ITS2 and *trnH-psbA* for medicinal plants. The database is very useful for traditional medicines from China and can give identified results quickly. But it is probably inapplicable to overseas and folk medicines. Moreover, NCBI provides much more comprehensive identification but it includes some DNA sequences with misidentification (Bridge et al., 2003; Federhen, 2012). Meanwhile, intra-species genetic distance may be higher than inter-species genetic distance especially in some complex and widespread species. So species of the nearest match is not necessarily correct identification (de Boer et al., 2014; Heubl, 2010, 2013). Identification should be made manually taking E-value, maximum identify, number of closely related species represented in the database, as well as distribution of the plant in question into consideration.

There are still many problems to be solved for identifying herbal materials in market though DNA barcodes. Firstly, plant medicines from fern were difficult to be correctly identified, such as “Zhuzongcao” (QC002). DNA sequences of these groups were very absent and they were apt to be identified in error. These samples were generally identified in genus-specific level. Secondly, some genus is composed of multitudinous species and many species are used as medicines, such as *Artemisa* L.(Heubl, 2013). It is also difficult to identify herbal materials from these genera. Thirdly, some folk or local medicines could not be identified using DNA barcodes, especially using the DNA Barcoding System for Identifying Herbal Medicines. So we propose that relative organization and researchers should further supply DNA sequences for special groups and improving identification of submitted sequences.

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Table 2: Herbal materials used for identification though DNA barcodes in this study and their identified information (I).

Sample ID	Local name of traditional medicine	Putative scientific name of original plant	Identified result by ITS2	Identified result by *trnH-psbA*	Confirmed scientific name of original plant	Family name
QC002	Zhuzongcao	*Adiantum capillus-veneris* L.	No sequence	*Adiantum* L.	*Adiantum* L.	Adiantaceae
QC003**	Baihuasheshecao	*Hedyotis diffusa* Willd.	*Arenaria serpyllifolia* L.	*Arenaria serpyllifolia* L.	*Arenaria serpyllifolia* L.	Caryophyllaceae
QC004**	Qianzhenwanxiancao	*Stellaria yunnanensis* Franch.	*Wahlenbergia marginata* (Thunb.) A.DC	Failure	*Wahlenbergia marginata* (Thunb.) A.DC	Campanulaceae
QC005*	Cheqiancao	*Plantago asiatica* L.	*Plantago major* L.	*Plantago major* L.	*Plantago major* L.	Plantaginaceae
QC007	Biandaxiuqiu	*Hemiphragma heterophyllum* wall.	*Hemiphragma heterophyllum* wall.	Failure	*Hemiphragma heterophyllum* wall.	Scrophulariaceae
QC008	Tougucao	*Gaultheria leucocarpa* Bl.	*Gaultheria leucocarpa* Bl.	*Gaultheria* Kalm *ex* L.	*Gaultheria leucocarpa* Bl.	Ericaceae
QC009	Jinxiancao	*Lysimachia christinae* Hance	*Lysimachia* L.	*Lysimachia* L.	*Lysimachia* L.	Primulaceae
QC010	Shuizhuzong	*Adiantum* L.	No sequence	*Adiantum* L.	*Adiantum* L.	Adiantaceae
QC011	Guanyincao	*Reineckia carnea* Kunth	No sequence	*Reineckia carnea* Kunth	*Reineckia carnea* Kunth	Liliaceae
QC012	Huixincao	*Rhodobryum giganteum* Par.	*Rhodobryum* Schimp	*Rhodobryum giganteum* Par.	*Rhodobryum giganteum* Par.	Bryaceae
QC013	Luxiancao	*Pyrola decorate* H. Andr.	*Pyrola decorate* H. Andr.	*Pyrola* L.	*Pyrola decorate* H. Andr.	Pyrolaceae
QC014	Feixincao	*Botrychium lanuginosum* Wall.	No sequence	*Botrychium* Sw.	*Botrychium* Sw.	Botrychiaceae
QC015	Shiwei	*Pyrrosia petiolosa* (Christ) Ching	No sequence	*Pyrrosia petiolosa* (Christ) Ching	*Pyrrosia petiolosa* (Christ) Ching	Polypodiaceae
QC016	Yimucao	*Leonurus japonicas* Houtt.	*Leonurus japonicas* Houtt.	*Leonurus japonicas* Houtt.	*Leonurus japonicas* Houtt.	Lamiaceae
QC017	Huangjingzi	*Vitex negundo* L.	*Vitex negundo* L.	*Vitex negundo* L.	*Vitex negundo* L.	Verbenaceae
QC018	Bohe	*Mentha Canadensis* L.	*Mentha Canadensis* L.	*Mentha Canadensis* L.	*Mentha Canadensis* L.	Lamiaceae
QC019	Zuogushengjingcao	Unconfirmed	No sequence	*Tupistra wattii* (C.B.Clarke) Hook.	*Tupistra wattii* (C.B.Clarke) Hook.	Liliaceae
QC020	Banzhilian	*Scutellaria barbata* D. Don	*Scutellaria barbata* D. Don	*Scutellaria barbata* D. Don	*Scutellaria barbata* D. Don	Lamiaceae
QC021	Shijiaocao	*Boenninghausenia sessilicarpa* Levl.	Failure	Failure	Failure	-
QC022	Bianxu	*Polygonum aviculare* L.	*Polygonum* L.	No sequence	*Polygonum* L.	Polygonaceae
QC023	Sanxuedan	Unconfirmed	*Peperomia blanda* (Jacquin) Kunth	Failure	*Peperomia blanda* (Jacquin) Kunth	Piperaceae
QC024	Maticao (Jinxiancao)	Unconfirmed	*Centella asiatica* (L.) Urban	*Centella asiatica* (L.) Urban	*Centella asiatica* (L.) Urban	Apiaceae
QC025**	Muguajisheng	*Scurrula parasitica* L.	Failure	*Taxillus chinensis* (DC) Danser	*Taxillus chinensis* (DC) Danser	Loranthaceae

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Table 2: Herbal materials used for identification though DNA barcodes in this study and their identified information (II).

Sample ID	Local name of traditional medicine	Putative scientific name of original plant	Identified result by ITS2	Identified result by *trnH-psbA*	Confirmed scientific name of original plant	Family name
QC026	Luticao	*Pyrola decorate* Andr.	*Pyrola decorate* Andr.	*Pyrola* L.	*Pyrola decorate* Andr.	Pyrolaceae
QC027	Xiakucao	*Prunella vulgaris* L.	*Prunella vulgaris* L.	*Prunella vulgaris* L.	*Prunella vulgaris* L.	Lamiaceae
QC028	Longdancao	*Gentiana rigescens* Franch.	*Gentiana rigescens* Franch.	*Gentiana rigescens* Franch.	*Gentiana rigescens* Franch.	Gentianaceae
QC029 **	Muguajisheng	*Scurrula parasitica* L.	Failure	*Taxillus chinensis* (DC) Danser	*Taxillus chinensis* (DC) Danser	Loranthaceae
QC030	Pugongying	*Taraxacum mongolicum* Han.-Mazz.	*Taraxacum mongolicum* Han.-Mazz.	*Taraxacum mongolicum* Han.-Mazz.	*Taraxacum mongolicum* Han.-Mazz.	Asteraceae
QC031	Yinchenhao	*Artemisia capillaris* Thunb.	*Artemisia capillaris* Thunb.	*Artemisia capillaris* Thunb.	*Artemisia capillaris* Thunb.	Asteraceae
QC032 *	Aiye	*Artemisia argyi* Levl. *et* Van.	*Artemisia lavandulaefolia* DC. Prodr.	*Artemisia* L.	*Artemisia lavandulaefolia* DC. Prodr.	Asteraceae
QC033	Xiakucao	*Prunella vulgaris* L.	*Prunella vulgaris* L.	*Prunella vulgaris* L.	*Prunella vulgaris* L.	Lamiaceae
QC034	Shiwei	*Pyrrosia* Mirbel	No sequence	*Pyrrosia petiolosa* (Christ) Ching	*Pyrrosia petiolosa* (Christ) Ching	Polypodiaceae
QC035	Yinyanghuo	*Epimedium* L.	*Epimedium* L.	*Epimedium* L.	*Epimedium* L.	Berberidaceae
QC036	Jingjie	*Nepeta tenuifolia* Benth.	*Nepeta tenuifolia* Benth.	*Nepeta tenuifolia* Benth.	*Nepeta tenuifolia* Benth.	Lamiaceae
QC037**	Yinyanghuo	*Epimedium* L.	*Astilbe rivularis* Buch.-Ham. *ex* D.Don	*Astilbe rivularis* Buch.-Ham.*ex*D.Don	*Astilbe rivularis* Buch.-Ham. *ex* D.Don	Saxifragaceae
QC038	Yimucao	*Leonurus japonicus* Houtt.	*Leonurus japonicus* Houtt.	*Leonurus japonicus* Houtt.	*Leonurus japonicus* Houtt.	Lamiaceae
QC039	Yexiahua	*Ainsliaea pertyoides* Franch.	*Ainsliaea pertyoides* Franch.	Faliure	*Ainsliaea pertyoides* Franch.	Asteraceae
QC040 *	Rendongteng	*Lonicera japonica* Thunb.	*Lonicera macranthoides* Hand.-Mazz.	*Lonicera* L.	*Lonicera macranthoides* Hand.-Mazz.	Caprifoliaceae
QC041	Dacheqian	*Plantago major* L.	*Plantago major* L.	*Plantago major* L.	*Plantago major* L.	Asteraceae
QC042	Fengweicao	*Pteris nervosa* Thunb.	No sequence	*Pteris* L.	*Pteris* L.	Pteridaceae
QC043	Maweihuanglianye	*Thalictrum* L.	*Thalictrum* L.	*Thalictrum* L.	*Thalictrum* L.	Ranunculaceae
QC044 *	Juanbai	*Selaginella tamariscina* Spring	*Selaginella pulvinata* Maxim.	*Selaginella pulvinata* Maxim.	*Selaginella pulvinata* Maxim.	Selaginellaceae
QC045	Daogouci	*Rubus delavayi* Franch.	*Rubus* L.	*Rubus* L.	*Rubus* L.	Rosaceae
QC046	Litoucao	*Viola inconspicua* Bl.	*Viola philippica* Cav.	*Viola philippica* Cav.	*Viola inconspicua* Bl.	Violaceae
QC047	Xianhecao	*Agrimonia pilosa* Ledeb.	*Agrimonia pilosa* Ledeb.	*Agrimonia* L.	*Agrimonia pilosa* Ledeb.	Rosaceae
QC048	Jinwaer(Waercao)	*Carpesium divaricatum* Sieb. *et* Zucc.	*Carpesium divaricatum* Sieb. *et* Zucc.	*Carpesium divaricatum* Sieb. *et* Zucc.	*Carpesium divaricatum* Sieb. *et* Zucc.	Asteraceae

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Table 2: Herbal materials used for identification though DNA barcodes in this study and their identified information (III).

Sample ID	Local name of traditional medicine	Putative scientific name of original plant	Identified result by ITS2	Identified result by *trnH-psbA*	Confirmed scientific name of original plant	Family name
QC049	Tiexianjue	*Adiantum* L.	No sequence	*Cheilanthes* Sw.	*Cheilanthes* Sw.	Sinopteridaceae
QC050*	Baihao	*Artemisia sieversiana* Ehrh. *ex* Willd.	*Artemisia argyi* Levl. *et* Van.	*Artemisia* L.	*Artemisia argyi* Levl. *et* Van.	Asteraceae
QC051	Xiangyejisheng	Unconfirmed	No sequence	Failure	Failure	-
QC052*	Jinyinhua	*Lonicera japonica* Thunb.	*Lonicera macranthoides* Hand.-Mazz.	*Lonicera* L.	*Lonicera macranthoides* Hand.-Mazz.	Caprifoliaceae
QC053	Cujiangcao	*Oxalis corniculata* L.	*Oxalis corniculata* L.	*Oxalis corniculata* L.	*Oxalis corniculata* L.	Oxalidaceae
QC054	Xiaofengweicao	*Asplenium* L.	No sequence	*Asplenium* L.	*Asplenium* L.	Aspleniaceae
QC055	Guizhencao	*Bidens pilosa* L.	*Bidens pilosa* L.	*Bidens* L.	*Bidens pilosa* L.	Asteraceae
QC056	Liangmianzhen	*Solanum torvum* Sw.	No sequence	*Solanum torvum* Sw.	*Solanum torvum* Sw.	Solanaceae
QC057	Heihanlian	*Eclipta prostrate* L.	*Eclipta prostrate* L.	*Eclipta prostrate* L.	*Eclipta prostrate* L.	Asteraceae
QC058	Laoguancao	*Geranium nepalense* Sw.	*Geranium nepalense* Sw.	*Geranium nepalense* Sw.	*Geranium nepalense* Sw.	Geraniaceae
QC059	Kudingding	Unconfirmed	*Solanum nigrum* L.	*Solanum nigrum* L.	*Solanum nigrum* L.	Solanaceae
QC060**	Wuzhuajinlong	*Cayratia japonica* (Thunb.) Gagnep.	*Tetrastigma* (Miq.) Planch.	*Tetrastigma* (Miq.) Planch.	*Tetrastigma* (Miq.) Planch.	Vitaceae
QC061	Pahao	*Elsholtzia rugulosa* Hemsl.	*Elsholtzia* Willd.	*Elsholtzia* Willd.	*Elsholtzia* Willd.	Lamiaceae
QC062	Zhuzongcao	*Adiantum* L.	No sequence	*Adiantum* L.	*Adiantum* L.	Adiantaceae
QC063	Jiejiecao	*Equisetum ramosissimum* Desf.	No sequence	No sequence	No sequence	-
QC064	Xixiancao	*Siegesbeckia orientalis* L.	No sequence	*Siegesbeckia orientalis* L.	*Siegesbeckia orientalis* L.	Asteraceae
QC065	Digandou	*Rorippa indica* (L.) Hiern	*Rorippa indica* (L.) Hiern	Failure	*Rorippa indica* (L.) Hiern	Brassicaceae
QC066	Dengxincao	*Juncus effuses* L.	No sequence	No sequence	No sequence	-
QC067	Chaihu	*Bupleurum* L.	*Bupleurum chinense* DC. Prodr.	*Bupleurum* L.	*Bupleurum chinense* DC. Prodr.	Apiaceae
QC068	Xixin	*Asarum himalaicum* Hook. *et* Thoms.	*Asarum himalaicum* Hook. *et* Thoms	No sequence	*Asarum himalaicum* Hook. *et* Thoms	Aristolochiaceae
QC069	Yinchenhao	*Artemisia capillaris* Thunb.	*Artemisia capillaris* Thunb.	*Artemisia capillaris* Thunb.	*Artemisia capillaris* Thunb.	Asteraceae
QC070	Xuelian	*Saussurea* DC.	*Saussurea medusa* Maxim.	*Saussurea* DC.	*Saussurea medusa* Maxim.	Asteraceae
QC071*	Jinyinhua	*Lonicera japonica* Thunb.	*Lonicera macranthoides* Hand.-Mazz.	*Lonicera macranthoides* Hand.-Mazz.	*Lonicera macranthoides* Hand.-Mazz.	Caprifoliaceae

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Table 2 Herbal materials used for identification though DNA barcodes in this study and their identified information (IV).

Sample ID	Local name of traditional medicine	Putative scientific name of original plant	Identified result by ITS2	Identified result by *trnH-psbA*	Confirmed scientific name of original plant	Family name
QC072	Yuxingcao	*Houttuynia cordata* Thunb.	*Houttuynia cordata* Thunb.	*Houttuynia cordata* Thunb.	*Houttuynia cordata* Thunb.	Saururaceae
QC073	Chaihu (Wild)	*Bupleurum marginatum* Wall. ex DC.	*Bupleurum* L.	*Bupleurum* L.	*Bupleurum* L	Apiaceae
QC075*	Jinyinhua	*Lonicera japonica* Thunb.	*Lonicera macranthoides* Hand.-Mazz.	*Lonicera* L.	*Lonicera macranthoides* Hand.-Mazz.	Caprifoliaceae
QC076	Shihu	*Dendrobium nobile* Lindl.	No sequence	No sequence	No sequence	-
QC077	Qinghao	*Artemisia annua* L.	*Artemisia* L.	*Artemisia* L.	*Artemisia* L.	Asteraceae
QC079**	Celan	*Eupatorium fortune* Turcz.	*Polygonum chinense* L.	*Polygonum chinense* L.	*Polygonum chinense* L.	Polygonaceae
QC080	Jinzhongyinchen	*Siphonostegia chinensis* Benth.	No sequence	Failure	Failure	-
QC081	Zihuadiding	*Viola philippica* Cav.	*Viola philippica* Cav.	*Viola philippica* Cav.	*Viola philippica* Cav.	Violaceae
QC082	Xiangye	*Laurus nobilis* L.	Failure	*Laurus nobilis* L.	*Laurus nobilis* L.	Lauraceae
QC083*	Nanxixin (Tuxixin)	*Asarum forbesii* Maxim.	*Asarum himalaicum* Hook. *et* Thoms.	No sequence	*Asarum himalaicum* Hook. *et* Thoms.	Aristolochiaceae
QC084	Xiaofeixincao	Unconfirmed	No sequence	*Botrychium* Sw.	*Botrychium* Sw.	Botrychiaceae
QC085	Shexucao	*Ophioglossum* L.	No sequence	*Ophioglossum* L.	*Ophioglossum* L.	Ophioglossaceae
QC086	Dengzhanhua	*Erigeron breviscapus* Hand.-Mazz.	*Erigeron breviscapus* Hand.-Mazz.	*Erigeron* L.	*Erigeron breviscapus* Hand.-Mazz.	Asteraceae
QC087	Jiaogulan	*Gynostemma pentaphyllum* Makino	No sequence	*Gynostemma pentaphyllum* Makino	*Gynostemma pentaphyllum* Makino	Cucurbitaceae
QC088	Banzhilian	*Scutellaria barbata* D. Don	*Scutellaria barbata* D. Don	*Scutellaria barbata* D. Don	*Scutellaria barbata* D. Don	Lamiaceae
QC089	Sheyancao	*Saussurea romuleifolia* Franch.	*Saussurea* DC.	*Saussurea* DC.	*Saussurea* DC.	Asteraceae
QC090	Xiaoshancha	*Elsholtzia bodinieri* Vaniot	Failure	*Elsholtzia* Willd.	*Elsholtzia* Willd.	Lamiaceae
QC091	Sanxuedan	*Peperomia blanda* (Jacquin) Kunth	No sequence	Failure	Failure	-
QC092	Guichuixiao	*Leycesteria Formosa* Wall.	Failure	Failure	Failure	-
QC093**	Lvticao	*Caltha palustris* L.	*Centella asiatica* (L.) Urban	*Centella asiatica* (L.) Urban	*Centella asiatica* (L.) Urban	Apiaceae
QC094**	Cebaiye	*Platycladus orientalis* (L.) Franco	No sequence	*Lycopodium japonicum* Thunb.	*Lycopodium japonicum* Thunb.	Lycopodiaceae
QC095	Aiye	*Artemisia argyi* Levl. *et* Van.	*Artemisia argyi* Levl. *et* Van.	*Artemisia* L.	*Artemisia argyi* Levl. *et* Van.	Asteraceae

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Table 2: Herbal materials used for identification though DNA barcodes in this study and their identified information (V).

Sample ID	Local name of traditional medicine	Putative scientific name of original plant	Identified result by ITS2	Identified result by *trnH-psbA*	Confirmed scientific name of original plant	Family name
QC096*	Choulingdan	*Laggera pterodonta* (DC.) Benth.	*Laggera pterodonta* (DC.) Benth.	*Laggera pterodonta* (DC.) Benth.	*Laggera pterodonta* (DC.) Benth.	Asteraceae
QC097	Xianglingcao	*Crotalaria ferruginea* Grah. *ex* Benth.	*Crotalaria* L.	*Crotalaria* L.	*Crotalaria* L.	Fabaceae
QC098	Longdancao	*Gentiana rigescens* Franch.	*Gentiana rigescens* Franch.	*Gentiana rigescens* Franch.	*Gentiana rigescens* Franch.	Gentianaceae
QC099	Longdancao	*Gentiana rigescens* Franch.	*Gentiana rigescens* Franch.	*Gentiana rigescens* Franch.	*Gentiana rigescens* Franch.	Gentianaceae
QC100**	Sifanghao	*Elsholtzia blanda* Benth.	*Salvia plebeia* R. Br.	*Salvia plebeia* R. Br.	*Salvia plebeia* R. Br.	Lamiaceae
QC101	Dafeixincao	Unconfirmed	No sequence	*Botrychium* Sw.	*Botrychium* Sw.	Botrychiaceae
QC102	Tianjihuang	Unconfirmed	Failure	No sequence	Failure	-
QC103	Gangbangui	*Polygonum perfoliatum* L.	*Polygonum perfoliatum* L.	*Polygonum* L.	*Polygonum perfoliatum* L.	Polygonaceae
QC104**	Baitouweng	*Pulsatilla chinensis* (Bunge) Regel	*Carpesium divaricatum* Sieb. *et* Zucc.	*Carpesium* L.	*Carpesium divaricatum* Sieb. *et* Zucc.	Asteraceae
QC106**	Xuelimei	*Gentiana rhodantha* Franch.	*Metagentiana rhodantha* T.N. Ho	Failure	*Metagentiana rhodantha* T.N. Ho	Gentianaceae
QC107**	Xiangru	*Elsholtzia ciliate* (Thunb.) Hyland	*Origanum vulgare* L.	*Origanum vulgare* L.	*Origanum vulgare* L.	Lamiaceae
QC108	Dashiwei	*Pyrrosia* Mirbel	No sequence	*Pyrrosia* Mirbel	*Pyrrosia* Mirbel	Polypodiaceae
QC109**	Xiangrucao	*Elsholtzia ciliate* (Thunb.) Hyland	*Origanum vulgare* L.	*Origanum vulgare* L.	*Origanum vulgare* L.	Lamiaceae
QC110	Xianglingcao	*Crotalaria ferruginea* Grah. ex Benth.	Failure	Failure	Failure	-
QC111	Zisu	*Perilla frutescens* (L.) Britt.	*Perilla frutescens* (L.) Britt.	*Perilla frutescens* (L.) Britt.	*Perilla frutescens* (L.) Britt.	Lamiaceae
QC112*	Qingyedan	*Swertia mileensis* T.N.Ho et W. L. Shi	*Swertia macrosperma* C.B. Clarke	*Swertia macrosperma* C.B. Clarke	*Swertia macrosperma* C.B. Clarke	Gentianaceae
QC113	Huashicao	*Pyrrosia* Mirbel.	No sequence	Failure	Failure	-
QC114	Hanzhuzongcao	*Adiantum* L.	Failure	Failure	Failure	-
QC115	Fanxieye	*Senna* Miller	*Senna* Miller	*Senna* Miller	*Senna* Miller	Fabaceae
QC116*	Qumai	*Dianthus superbus* L.	*Dianthus chinensis* L.	*Dianthus chinensis* L.	*Dianthus chinensis* L.	Caryophyllaceae

Note: * substitute of herbal material; ** adulterant.

Conclusions

In the present study, DNA barcodes showed great identified power for whole herb. ITS2 possessed better discriminatory ability than *trnH-psbA* region but its success rates of PCR amplification and DNA sequencing were obviously lower. Both ITS2 and *trnH-psbA* should be listed as standard regions of DNA barcodes for herbal medicines. The DNA Barcoding System for Identifying Herbal Medicine is an efficient identified platform for Chinese traditional medicines. But DNA sequences of the folk medicines should be further supplemented and improved. Moreover, reference databases, such as NCBI, must construct error-corrected mechanism that eliminates DNA sequences with mistaken identification (Heubl, 2013). Although molecular identification often fails to assign individuals to species it is a helpful tool in providing clues for identifying herbal materials that lack morphological features for species identification.

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