Chinese Herbal Medicines 11 (2019) 275–280

Contents lists available at ScienceDirect

Chinese Herbal Medicines

journal homepage: www.elsevier.com/locate/chmed

Original Article

Identification of flower herbs in Chinese pharmacopoeia based on DNA barcoding

Xue-min Wei, Xiao-yue Wang, Zi-tong Gao, Pei Cao, Jian-ping Han*

Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing 100193, China

ARTICLE INFO

Article history:
Received 19 November 2018
Revised 13 March 2019
Accepted 21 March 2019
Available online 24 May 2019

Keywords:
adulteration
flower herbs
healthcare teas
ITS2
survey

ABSTRACT

Objective: Flower herbs are an important category of traditional Chinese medicinal materials, some of which are used as healthcare tea in folk. However, the increasing adulteration of medicinal herbs is threatening consumer safety. The adulteration of flower herbs and their healthcare tea products in the market were investigated.

Methods: A total of 33 flower herb samples from several retail pharmacies in China were randomly collected and 27 flower healthcare tea samples were purchased online. They were identified using ITS2-based Traditional Chinese Medicine Database (TCMD). Additionally, *Lonicerae Japonicae Flos* and the adulterants were compared in the ITS2 secondary structures.

Results: There were one adulterant (*Inulae Flos*) in flower herb materials and eight adulterants in healthcare tea samples. *Inula linariifolia* was an adulterate species of *Inulae Flos, Robinia pseudoacacia* was of *Sophorae Flos*, and *Lonicera macranthoides* was of *Lonicerae Japonicae Flos. Sophorae Flos* and *Lonicerae Japonicae Flos* were two healthcare tea products with high adulteration rates.

Conclusion: The TCMD is powerful tool to identify flower herbs and the adulterants that frequently occurred in the flower herb market, especially online shops.

© 2019 Tianjin Press of Chinese Herbal Medicines. Published by Elsevier B.V. All rights reserved.

1. Introduction

Flower herbs largely include medicinal materials from the flower, pollen, spica, calyx, stamen and stigma of medicinal plants. There are 31 flower herbs in Chinese Pharmacopoeia (Pharmacopoeia Committee of P. R. China, 2015), such as *Farfarae Flos* (Kuandonghua in Chinese), *Croci Stigma* (Xihonghua in Chinese), *Lonicerae Japonicae Flos* (Jinyinhua in Chinese), *Albiziae Flos* (Hehuanhua in Chinese), and *Typhae Pollen* (Puhuang in Chinese), playing a key role in modern medicinal and health services (Liu, Chuang, Lam, Jiang, & Cheng, 2015; Xiao, Yan, Chen, & Zhou, 2015). However, the adulteration of medicinal herbs has developed into a serious problem in the market (De Smet, 2004; Jiang et al., 2016). For example, the substitution of *Campsis Flos* (Lingxiaohua in Chinese) by flowers of *Datura* species has become the most common cause of Chinese herbal medicine-induced anticholinergic poisoning in Hong Kong, China (Cheng, Chan, Mak, Tse, & Lau, 2013). Traditional techniques including morphological, microscopic, and chemical identification are not accurate for some flower drugs. A study showed that the average fineness of several typical Chinese medicinal materials processed by ultrafine grinding technology was 2.0 – 12.7 microns (Jing, 2005). Therefore, morphological and microscopic identification cannot be adopted because identifiable features of medicinal materials were lost during processing.

DNA barcoding was first proposed by Herbert in 2003, allowing for species identification without specialized taxonomic knowledge (Hebert, Cywinska, Ball, & deWaard, 2003; Kress, Wurdack, Zimmer, Weigt, & Janzen, 2005; Schindel, & Miller, 2005) . The second internal transcribed spacer (ITS2) sequence is used as a standard DNA barcode for the identification of medicinal plants and their closely related species (Chiou, Yen, Fang, Chen, & Lin, 2007; Liu et al., 2018; Yao et al., 2010). The Traditional Chinese Medicine Database (TCMD) is a traditional Chinese medicine barcode platform that was established by our group using an ITS2+*psbA-trnH* two loci barcode combination (Kress, & Erickson, 2007). It is the largest DNA barcode database of medicinal materials in the world (Chen et al., 2014) including flower herbs, comprising of 78 847 barcodes belonging to 23 262 medicinal species listed in Pharmacopoeias all over the world, such as Chinese, European, Korean and American (Han et al., 2016) and accessed at http://www.tcmbarcode.cn/china/. In addition, the ITS2 secondary structures are an available tool for species identification (Grajales, Aguilar, & Sánchez, 2007). And all the secondary structures of complete ITS2 sequences with GI numbers in GenBank could be predicted by the ITS2 database (Schultz et al., 2006).

* Corresponding author.
E-mail address: jphan@implad.ac.cn (J.-p. Han).

https://doi.org/10.1016/j.chmed.2019.05.003
1674-6384/© 2019 Tianjin Press of Chinese Herbal Medicines. Published by Elsevier B.V. All rights reserved.

Cortex herbs and herbal herbs of the Chinese Pharmacopoeia have been successfully identified using ITS2 barcodes in previous studies (Pang, Shi, Song, Chen, & Chen, 2013; Sun, & Chen, 2013). However, these studies have focused on neither the authentication of flower herbs of Chinese Pharmacopoeia nor using the ITS2-based TCMD. Here, we investigated the adulteration of flower herbs and healthcare tea in the market which is help to guide proper consumption. Additionally, the secondary structures of ITS2 regions were predicted to identify *Lonicerae Japonicae Flos* and its adulterants.

2. Materials and methods

2.1. Sampling

A total of 60 flower herb products were collected in our study, and they consisted of medicinal materials and correlated tea products. Of these products, 33 samples (S1−S33) were randomly collected from retail pharmacy in Beijing, China (Table 1, Fig. 1) and represented 20 types of flower herbs recorded in the Chinese Pharmacopoeia. In addition, 27 samples (S34−S60) were purchased online, and they represented seven major flower healthcare tea products available in the markets. All the samples were dried in silica gel, and voucher samples were deposited in the herbarium of Institute of Medicinal Plant Development.

2.2. DNA extraction, amplification and sequencing

Twenty milligrams of each sample were rubbed for 2 min at a frequency of 30 times/s in a Fast–Prep bead mill (Retsch MM400, Germany). DNA extraction was performed using the Plant Genomic DNA Kit (Tiangen Biotech Beijing Co., China) according to the manufacturer's instructions. The universal primer pair 2F (5′-ATGCGATACTTGGTGTGAAT-3′)/3R (5′-GACGCTTCTCCAGACTACAAT-3′) was used for ITS2 amplification (Chen et al., 2010). Polymerase chain reaction (PCR) amplification in a 25 μL volume was performed according to the procedure provided by Chen et al. (2013). The purified PCR products were sequenced by the Major Engineering Laboratory of the Chinese Academy of Agricultural Sciences University. All purified PCR products were sequenced in both the forward and reverse directions.

2.3. Data analysis

The sequences obtained from the experiment were assembled using CodonCode Aligner 6.0.2, and the primer sequences and low-quality regions at both ends of the sequencing results were removed. The sequences were then annotated and delimited using the Hidden Markov Model (HMM) (Keller et al., 2009) method to remove the conserved 5.8S and 28S rDNA regions, and they were then deposited in the TCMD for identification based on the Basic Local Alignment Search Tool (BLAST) method. ITS2 secondary structures were predicted with the ITS2 database website: http://its2.bioapps.biozentrum.uni–wuerzburg.de/.

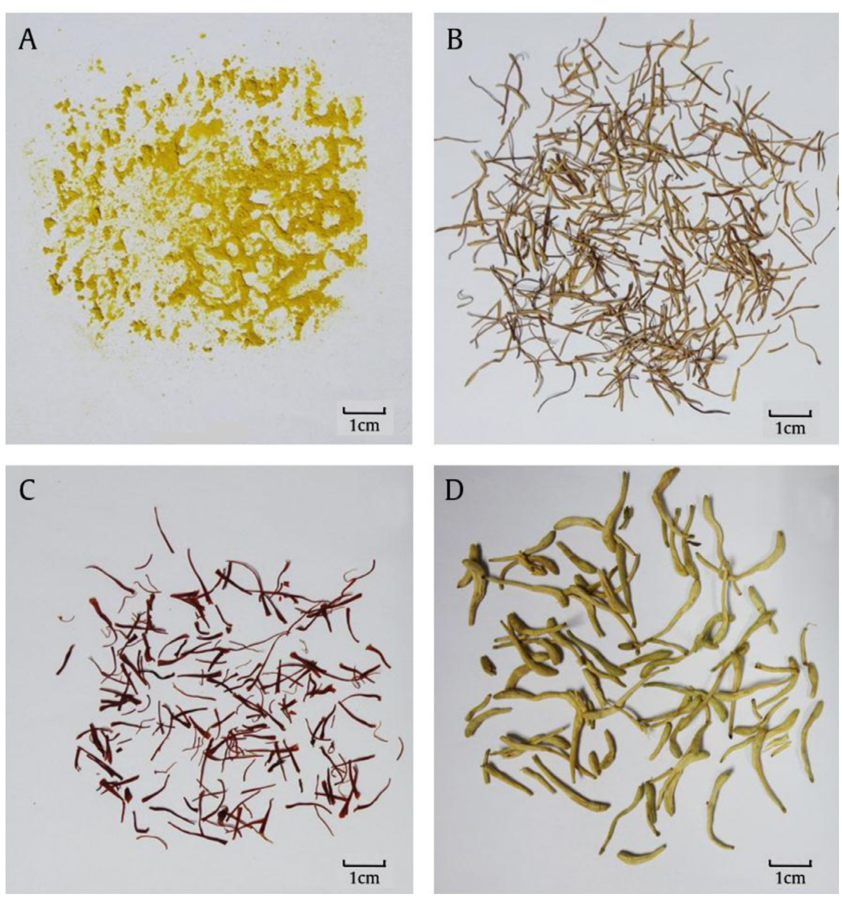

Fig. 1. Four commercial flower herb samples (A: S13, *Typhae Pollen*; B: S16, *Nelumbinis Stamen*; C: S31, *Croci Stigma*; and D: S10, *Lonicerae Japonicae Flos*) that were difficult to be determined based on traditional methods.

Table 1
Sixty samples and their identification results in detail.

Sample No.	Latin names of medicinal materials	Chinese names	Latin names of original species	Identification results	Genuine or not
S1	*Schizonepetae Spica*	Jingjiesui	*Schizonepeta tenuifolia* Briq.	*S. tenuifolia*	Genuine
S2	*Campsis Flos*	Lingxiaohua	*Campsis grandiflora* (Thunb.) K. Schum.; *Campsis radicans* (L.) Seem.	*C. radicans*	Genuine
S3	*Physalis Calyx Seu Fructus*	Jindenglong	*Physalis alkekengi* L. var. *franchetii* (Mast.) Makino	*P. alkekengi*	Genuine
S4	*Buddlejae Flos*	Mimenghua	*Buddleja officinalis* Maxim.	*B. officinalis*	Genuine
S5	*Carthami Flos*	Honghua	*Carthamus tinctorius* L.	*C. tinctorius*	Genuine
S6	*Carthami Flos*	Honghua	*C. tinctorius*	*C. tinctorius*	Genuine
S7	*Rosae Rugosae Flos*	Meiguihua	*Rosa rugosa* Thunb.	–	–
S8	*Rosae Chinensis Flos*	Yuejihua	*Rosa chinensis* Jacq.	–	–
S9	*Sophorae Flos*	Huaihua	*Sophora japonica* L.	*S. japonica*	Genuine
S10	*Lonicerae Japonicae Flos*	Jinyinhua	*Lonicera japonica* Thunb.	*L. japonica*	Genuine
S11	*Inulae Flos*	Xuanfuhua	*I. japonica; I. britannica*	*Inula linariifolia* Turcz.	Not
S12	*Mume Flos*	Meihua	*Prunus mume* (Sieb.) Sieb. et Zucc.	*P. mume*	Genuine
S13	*Typhae Pollen*	Puhuang	*Typha angustifolia* L.; *Typha orientalis* Presl	*T. orientalis*	Genuine
S14	*Kaki Calyx*	Shidi	*Diospyros kaki* Thunb.	*D. kaki*	Genuine
S15	*Chrysanthemi Indici Flos*	Yejuhua	*Chrysanthemum indicum* L.	–	–
S16	*Nelumbinis Stamen*	Lianxu	*Nelumbo nucifera* Gaertn.	*N. nucifera*	Genuine
S17	*Albiziae Flos*	Hehuanhua	*Albizia julibrissin* Durazz.	*A. julibrissin*	Genuine
S18	*Farfarae Flos*	Kuandonghua	*Tussilago farfara* L.	*T. farfara*	Genuine
S19	*Celosiae Cristatae Flos*	Jiguanhua	*Celosia cristata* L.	*C. cristata*	Genuine
S20	*Lonicerae Japonicae Flos*	Jinyinhua	*L. japonica*	*L. japonica*	Genuine
S21	*Rosae Rugosae Flos*	Meiguihua	*R. rugosa*	–	–
S22	*Chrysanthemi Indici Flos*	Yejuhua	*C. indicum*	–	–
S23	*Mume Flos*	Meihua	*P. mume*	*P. mume*	Genuine
S24	*Farfarae Flos*	Kuandonghua	*T. farfara*	*T. farfara*	Genuine
S25	*Lonicerae Japonicae Flos*	Jinyinhua	*L. japonica*	*L. japonica*	Genuine
S26	*Lonicerae Japonicae Flos*	Jinyinhua	*L. japonica*	*L. japonica*	Genuine
S27	*Lonicerae Japonicae Flos*	Jinyinhua	*L. japonica*	*L. japonica*	Genuine
S28	*Lonicerae Japonicae Flos*	Jinyinhua	*L. japonica*	*L. japonica*	Genuine
S29	*Lonicerae Japonicae Flos*	Jinyinhua	*L. japonica*	*L. japonica*	Genuine
S30	*Lonicerae Japonicae Flos*	Jinyinhua	*L. japonica*	*L. japonica*	Genuine
S31	*Croci Stigma*	Xihonghua	*Crocus sativus* L.	*C. sativus*	Genuine
S32	*Chrysanthemi Flos*	Juhua	*Chrysanthemum morifolium* Ramat.	–	–
S33	*Chrysanthemi Flos*	Juhua	*C. morifolium*	–	–
S34	*Croci Stigma*	Xihonghua	*C. sativus*	*C. sativus*	Genuine
S35	*Croci Stigma*	Xihonghua	*C. sativus*	*C. sativus*	Genuine
S36	*Croci Stigma*	Xihonghua	*C. sativus*	*C. sativus*	Genuine
S37	*Sophorae Flos*	Huaihua	*S. japonica*	*S. japonica*	Genuine
S38	*Sophorae Flos*	Huaihua	*S. japonica*	*Robinia pseudoacacia* Linn	Not
S39	*Sophorae Flos*	Huaihua	*S. japonica*	*R. pseudoacacia*	Not
S40	*Sophorae Flos*	Huaihua	*S. japonica*	*R. pseudoacacia*	Not
S41	*Sophorae Flos*	Huaihua	*S. japonica*	*R. pseudoacacia*	Not
S42	*Nelumbinis Stamen*	Lianxu	*N. nucifera*	*N. nucifera*	Genuine
S43	*Nelumbinis Stamen*	Lianxu	*N. nucifera*	*N. nucifera*	Genuine
S44	*Nelumbinis Stamen*	Lianxu	*N. nucifera*	*N. nucifera*	Genuine
S45	*Inulae Flos*	Xuanfuhua	*I. japonica; I. britannica*	*I. britannica*	Genuine
S46	*Inulae Flos*	Xuanfuhua	*I. japonica; I. britannica*	*I. japonica*	Genuine
S47	*Inulae Flos*	Xuanfuhua	*I. japonica; I. britannica*	*I. japonica*	Genuine
S48	*Inulae Flos*	Xuanfuhua	*I. japonica; I. britannica*	*I. linariifolia*	Not
S49	*Inulae Flos*	Xuanfuhua	*I. japonica; I. britannica*	*I. japonica*	Genuine
S50	*Lonicerae Japonicae Flos*	Jinyinhua	*L. japonica*	*L. japonica*	Genuine
S51	*Lonicerae Japonicae Flos*	Jinyinhua	*L. japonica*	*Lonicera macranthoides* Hand. -Mazz	Not
S52	*Lonicerae Japonicae Flos*	Jinyinhua	*L. japonica*	*L. macranthoides*	Not
S53	*Lonicerae Japonicae Flos*	Jinyinhua	*L. japonica*	*L. japonica*	Genuine
S54	*Lonicerae Japonicae Flos*	Jinyinhua	*L. japonica*	*L. macranthoides*	Not
S55	*Albiziae Flos*	Hehuanhua	*A. julibrissin*	*A. julibrissin*	Genuine
S56	*Albiziae Flos*	Hehuanhua	*A. julibrissin*	*A. julibrissin*	Genuine
S57	*Albiziae Flos*	Hehuanhua	*A. julibrissin*	*A. julibrissin*	Genuine
S58	*Physalis Calyx Seu*	Jindenglong	*P. alkekengi*	*P. alkekengi*	Genuine
S59	*Physalis Calyx Seu*	Jindenglong	*P. alkekengi*	*P. alkekengi*	Genuine
S60	*Physalis Calyx Seu*	Jindenglong	*P. alkekengi*	*P. alkekengi*	Genuine

Note: "–" represents could not be determined.

3. Results

3.1. Nine adulterants in market identified by TCMD database

For the 60 flower herb samples, the PCR and sequencing success rate was 100%. A BLAST search was performed to estimate the reliability of species identification by the TCMD (Chen et al., 2014). All 60 ITS2 sequences generated in our study were deposited to TCMD for BLAST identification (Table 1). Of the 33 samples collected from retail pharmacy, one (label species: *Inulae Flos*, Xuanfuhua) was an adulterant and seven could not be determined, meaning that the best BLAST hits for a query sequence covered several species, including the target species. These seven samples belonged to four types of flower herbs including *Chrysanthemum Morifolium Flos* (Juhua in Chinese), *Chrysanthemum Indicum Flos* (Yejuhua in Chinese), *Rosa Rugosa Flos* (Meiguihua in Chinese), and *Rosa Chinensis Flos* (Yuejihua in Chinese). The BLAST results for the remaining samples were consistent with the commodity label species (Fig. 2).

Of the 27 ITS2 sequences generated from the healthcare tea samples purchased online, approximately 29.6% were adulterants. Three types of flower herbs, i.e., *Sophorae Flos* (Huaihua), *Inulae Flos* (Xuanfuhua), and *Lonicerae Japonicae Flos* (Jinyinhua) were not in accordance with the labeled species. Of the five *Flos Sophorae* tea samples, four samples were adulterate species *R. pseudoacacia*. One of the five *Flos Inulae* tea samples was adulterate species *I. linariifolia*. Three of the five *Flos Lonicerae Japonicae* tea samples were adulterate species *L. macranthoides*. The identification results of the rest samples were in accordance with the commercial names (Fig. 3).

In this study, of the 60 ITS2 sequences generated from the flower herb samples, only seven could not be identified using the TCMD; Thus, the identification efficiency was approximately 88.33%.

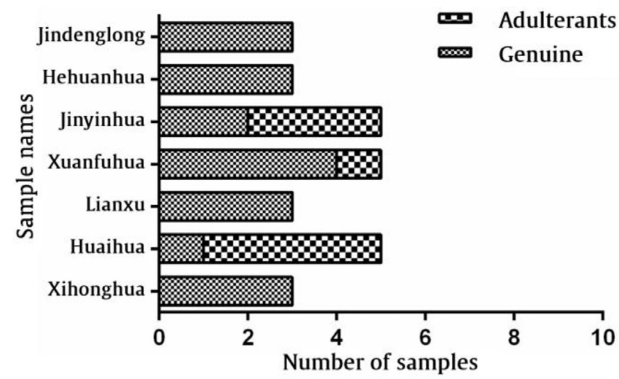

Fig. 3. Identification for seven major healthcare tea samples purchased online. All tea samples could be determined. One of five Xuanfuhua, three of five Jinyinhua and four of five Huaihua tea samples were adulterants.

tures of ITS2 regions of these species were predicted with the ITS2 database website: http://its2.bioapps.biozentrum.uni–wuerzburg.de/. *Lonicerae Japonicae Flos* and its adulterants exhibited the same typical four-helix folding consensus structure consisting of a central loop and four similar helices, namely, Helix I, II, III, and IV. Fig. 4 showed that the secondary structures of ITS2 regions among *L. japonica* and its adulterate species displayed significant differences in the four helices. The stem loop number, size and position on the helix I, II and III can differentiate *L. japonica* and its four adulterate species. Thus, the secondary structure is a candidate method to discriminate *Lonicerae Japonicae Flos* from its adulterants.

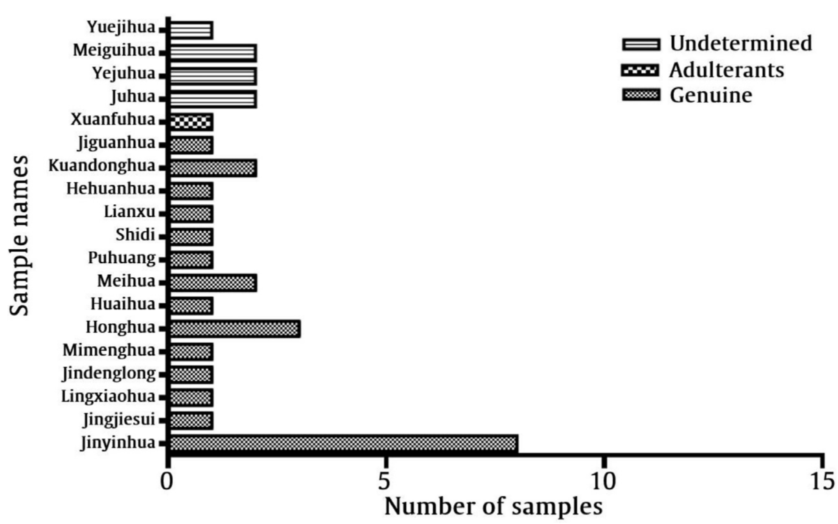

Fig. 2. Identification for 33 samples collected from retail pharmacies in China. One sample was an adulterant; Seven samples could not be determined; Remaining samples were genuine medicinal materials.

3.2. *Identification of* Lonicerae Japonicae Flos *and its adulterants by secondary structure*

As we known, *Lonicerae Japonicae Flos* is always confused with *Lonicerae Flos* (derived from *Lonicera macranthoides, Lonicera hypoglauca* Miq., *Lonicera confusa* DC., and *Lonicera fulvotomentosa* Hsu et S.C. Cheng). In our study, *L. Macranthoides* is also an adulterate species of *Lonicerae Japonicae Flos*. To distinguish *Lonicerae Japonicae Flos* from its adulterants, the secondary struc-

4. Discussion

4.1. TCMD is a powerful tool for detecting adulterants in flower herb marketplace

DNA molecular identification is an effective tool when traditional identification methods are not accurate. And several molecular plant identification platforms have been established. (De Boer, Ichim, & Newmaster, 2015; Heubl, 2010). For example,

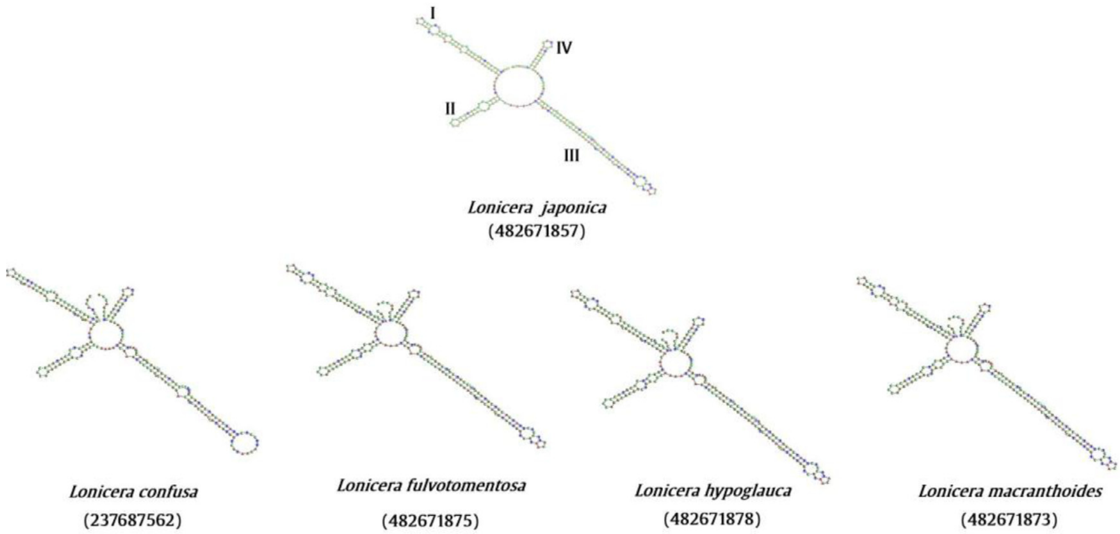

Fig. 4. ITS2 secondary structures for *L. japonica* and its adulterants. *L. Japonica* and its four adulterate species could be identified directly in terms of stem loop number, size and position on helixes.

the GenBank database (http://www.ncbi.nlm.nih.gov/genbank/) is frequently used for species identification. However, many medicinal sample sequences are not adequately represented in GenBank. TCMD is a traditional Chinese medicine barcode platform which contains 23 262 medicinal and closely related species, including adulterants and substitutions. As the largest DNA barcode database of medicinal materials in the world, it covers all the flower herbs listed in the Chinese Pharmacopoeia. Thus, the TCMD platform is the most suitable for the identification of flower herbs. In our study, the 60 samples collected from the market covered almost all the flower herbs in the Chinese Pharmacopoeia, except for a few toxic Chinese herbal medicines unavailable due to strict supervision in China, such as *Genkwa Flos* (Yuanhua in Chinese), *Daturae Flos* (Yangjinhua in Chinese), and *Rhododendri Mollis Flos* (Naoyanghua in Chinese). Our results showed that 88.33% of the total samples could be successfully identified using TCMD, and nine adulterants were successfully detected, indicating that TCMD has good ability for detecting the adulterants in flower herbs market. Furthermore, this article presents the first study of the systematic DNA barcode identification of flower medicinal materials in the Chinese Pharmacopoeia.

4.2. More caution should be focused on monitoring flower healthcare tea products

According to our study, the adulteration rate of the online market of flower healthcare tea was as high as 29.6%. Among the seven primary healthcare tea products we investigated, the adulteration rate of *Flos Sophorae* tea was the highest, up to 80%, followed by *Flos Lonicerae Japonicae* tea, and the adulteration rate was as high as 60%. In addition, the adulteration rate of *Flos Inulae* tea was 20%. Thus, the results revealed serious adulteration of flower healthcare tea products in the market, and we suggested that consumers should avoid the types of flower healthcare tea with high adulteration rates. Additionally, this survey also demonstrated that more caution should be focused on the flower herb market, especially online markets for flower healthcare tea.

4.3. Complete chloroplast genome sequence is suggested to be used in identification of related species

In our study, the adulterate species of *Sophorae Flos, Inulae Flos* and *Lonicerae Japonicae Flos* were accurately detected, they were *R. pseudoacacia*, *I. linariifolia* and *L. macranthoides*, respectively. *I. linariifolia* and *L. macranthoides* are significantly different in chemical composition and pharmacological bioactivities from *Inulae Flos* and *Lonicerae Japonicae Flos*, respectively (Chu, Liu, Qi, Liu, & Li, 2011; Seca, Grigore, Pinto, & Silva, 2014; Shi et al., 2013; Yuan, Wang, Jiang, Wang, & Huang, 2014). Additionally, *R. pseudoacacia* is a toxic species (Luo et al., 2016). Therefore, these adulterants should be accurately identified to ensure the safe use of flower herbs.

However, there are still seven samples that cannot be distinguished in this study. We suggest that the complete chloroplast genome sequence can be employed. The complete chloroplast genomes have shown to be highly effective for resolving relationships among species with low molecular divergence, and been used as an alternative to short diagnostic barcoding regions. And it has recently been widely used for the molecular identification of closely related species (Kang et al., 2017; Kuang et al., 2011; Moore, Soltis, Bell, Burleigh, & Soltis, 2010; Zhang et al., 2017). Moreover, other methods of the systems biology approach can work synergistically with DNA barcoding, such as transcriptomics and proteomics (Mishra et al., 2016).

5. Conclusion

In this study, we investigated 60 samples that represent 20 types of flower herbs recorded in the Chinese Pharmacopoeia and the related healthcare tea products. The TCMD played a key role in the accurate identification of flower herbs in this survey. Approximately 88.33% of the total samples could be successfully identified. This investigation surprisingly revealed a serious adulteration in the flower herb market. Nine samples were found to be substituted with other species, especially the following three flower herbs: *Sophorae Flos* (Huaihua in Chinese), *Inulae Flos* (Xuanfuhua in Chinese) and *Lonicerae Japonicae Flos* (Jinyinhua in Chinese). Most of these adulterants were healthcare tea samples purchased from online market. Flower healthcare teas with high adulteration rates were provided here, which can guide proper consumption.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

This work was supported by grants from the National Natural Science Foundation of China (no. 81673552) and CAMS Innovation Fund for Medical Sciences(CIFMS, 2016-I2M-3-016).

References

Chen, S. L., Yao, H., Han, J. P., Liu, C., Song, J. Y., Shi, L. C., et al. (2010). Validation of the ITS2 region as a novel DNA barcode for identifying medicinal plant species. *PLoS One, 5*(1), e8613.

Chen, S. L., Yao, H., Han, J. P., Xin, T. Y., Pang, X. H., Shi, L. C., et al. (2013). Principles for molecular identification of traditional Chinese materia medica using DNA barcoding. *China Journal of Chinese Materia Medica, 02*, 141–148.

Chen, S. L., Pang, X. H., Song, J. Y., Shi, L. C., Yao, H., Han, J. P., et al. (2014). A renaissance in herbal medicine identification: From morphology to DNA. *Biotechnology Advances, 32*(7), 1237–1244.

Cheng, K. L., Chan, Y. C., Mak, T. W., Tse, M. L., & Lau, F. L. (2013). Chinese herbal medicine-induced anticholinergic poisoning in Hong Kong. *Hong Kong Medical Journal, 19*(1), 38–41.

Chiou, S. J., Yen, J. H., Fang, C. L., Chen, H. L., & Lin, T. Y. (2007). Authentication of medicinal herbs using PCR-amplified ITS2 with specific primers. *Planta Medica, 73*(13), 1421–1426.

Chu, C., Liu, H. J., Qi, L. W., Liu, E. H., & Li, P. (2011). Combination of normal light and fluorescence microscopy for authentication of five *Lonicera* species flower buds. *Microscopy Research and Technique, 74*(2), 133–141.

De Boer, H. J., Ichim, M. C., & Newmaster, S. G. (2015). DNA barcoding and pharmacovigilance of herbal medicines. *Drug Safety, 38*(7), 611–620.

De Smet, P. A. (2004). Health risks of herbal remedies: An update. *Clinical Pharmacology & Therapeutics, 76*(1), 1–17.

Grajales, A., Aguilar, C., & Sánchez, J. A. (2007). Phylogenetic reconstruction using secondary structures of Internal Transcribed Spacer 2 (ITS2, rDNA): Finding the molecular and morphological gap in Caribbean gorgonian corals. *Bmc Evolutionary Biology, 7*(1), 90.

Han, J. P., Pang, X. H., Liao, B. S., Yao, H., Song, J. Y., & Chen, S. L. (2016). An authenticity survey of herbal medicines from markets in China using DNA barcoding. *Scientific Reports, 6*, 18723.

Hebert, P. D., Cywinska, A., Ball, S. L., & deWaard, J. R. (2003). Biological identifications through DNA barcodes. *Proceedings of the Royal society of London Biological Sciences, 270*(1512), 313–321.

Heubl, G. (2010). New aspects of DNA-based authentication of Chinese medicinal plants by molecular biological techniques. *Planta Medica, 76*(17), 1963–1974.

Jiang, C., Yuan, Y., Yang, G., Jin, Y., Liu, L. B., Zhao, Y. Y., et al. (2016). Fluorescence visual detection of herbal product substitutions at terminal herbal markets by CCP-based FRET technique. *Scientific Reports, 6*, 35540.

Jing, Y. E. (2005). Innovation study on Chinese medicinal materials ultrafine grinding by impacting and attriting in jet mill. *China Journal of Chinese Materia Medica, 30*(1), 27–29.

Kang, S. H., Lee, J. H., Lee, H. O., Ahn, B. O., Won, S. Y., Sohn, S. H., et al. (2017). Complete chloroplast genome and 45S nrDNA sequences of the medicinal plant species *Glycyrrhiza glabra* and *Glycyrrhiza uralensis*. *Genes & Genetic Systems, 93*(3). doi:10.1266/ggs.17-00002.

Keller, A., Schleicher, T., Schultz, J., Müller, T., Dandekar, T., & Wolf, M. (2009). 5.8S-28S rRNA interaction and HMM-based ITS2 annotation. *Gene, 430*(1–2), 50–57.

Kress, W. J., Wurdack, K. J., Zimmer, E. A., Weigt, L. A., & Janzen, D. H. (2005). Use of DNA barcodes to identify flowering plants. *Proceedings of the National Academy of Sciences of the United States of America, 102*(23), 8369–8374.

Kress, W. J., & Erickson, D. L. (2007). A two-locus global DNA barcode for land plants: The coding *rbcL* gene complements the non-coding *trnH-psbA* spacer region. *PLoS One, 2*(6), e508.

Kuang, D. Y., Wu, H., Wang, Y. L., Gao, L. M., Zhang, Z. S., & Lu, L. (2011). Complete chloroplast genome sequence of *Magnolia kwangsiensis* (Magnoliaceae): Implication for DNA barcoding and population genetics. *Genome, 54*(8), 663–673.

Liu, S. H., Chuang, W. C., Lam, W., Jiang, Z., & Cheng, Y. C. (2015). Safety surveillance of traditional Chinese medicine: Current and future. *Drug Safety, 38*(2), 117–128.

Liu, X. X., Ou, Q. M., Shi, Y. T., Xin, T. Y., Song, J. Y., Luo, J. J., et al. (2018). Identification of *Angelica sinensis* and its adulterants based on ITS2 sequence. *Chinese Traditional and Herbal Drugs, 49*(20), 4877–4883.

Luo, Y. J., Yuan, Y., Wang, R. Q., Liu, J., Du, N., & Guo, W. H. (2016). Functional traits contributed to the superior performance of the exotic species *Robinia pseudoacacia*: A comparison with the native tree *Sophora japonica*. *Tree Physiology, 36*(3), 345–355.

Mishra, P., Kumar, A., Nagireddy, A., Mani, D. N., Shukla, A. K., Tiwari, R., et al. (2016). DNA barcoding: An efficient tool to overcome authentication challenges in the herbal market. *Plant Biotechnology Journal, 14*(1), 8–21.

Moore, M. J., Soltis, P. S., Bell, C. D., Burleigh, J. G., & Soltis, D. E. (2010). Phylogenetic analysis of 83 plastid genes further resolves the early diversification of eudicots. *Proceedings of the National Academy of Sciences of the United States of America, 107*(10), 4623–4628.

Pang, X. H., Shi, L. C., Song, J. Y., Chen, X. C., & Chen, S. L. (2013). Use of the potential DNA barcode ITS2 to identify herbal materials. *Journal of Natural Medicines, 67*(3), 571–575.

Pharmacopoeia Committee of P. R. China. (2015). *Pharmacopoeia of the People's Republic of China* (pp. 30–354). Beijing, China: China Medical Science Press.

Schindel, D. E., & Miller, S. E. (2005). DNA barcoding a useful tool for taxonomists. *Nature, 435*(7038), 17.

Schultz, J., Müller, T., Achtziger, M., Seibel, P. N., Dandakar, T., & Wolf, M. (2006). The internal transcribed spacer 2 database–a web server for (not only) low level phylogenetic analyses. *Nucleic Acids Research, 34*(2), W704–W707.

Seca, A. M., Grigore, A., Pinto, D. C., & Silva, A. M. (2014). The genus *Inula* and their metabolites: From ethnopharmacological to medicinal uses. *Journal of Ethnopharmacology, 154*(2), 286–310.

Shi, X. W., Zhang, K., Xue, N., Su, L. F., Ma, G. X., Qi, J. L., et al. (2013). Differentiation of genuine *Inula britannica* L. and substitute specimens based on the determination of 15 components using LC-MS/MS and principal components analysis. *Food Chemistry, 141*(4), 4019–4025.

Sun, Z. Y., & Chen, S. L. (2013). Identification of cortex herbs using the DNA barcode nrITS2. *Journal of Natural Medicines, 67*(2), 296–302.

Xiao, F. L., Yan, B., Chen, L., & Zhou, D. (2015). Review of the use of botanicals for epilepsy in complementary medical systems–Traditional Chinese Medicine. *Epilepsy & Behavior, 52*(Pt B), 281–289.

Yao, H., Song, J. Y., Liu, C., Luo, K., Han, J. P., Li, Y., et al. (2010). Use of ITS2 region as the universal DNA barcode for plants and animals. *PLoS One, 5*(10), e13102.

Yuan, Y., Wang, Z. Y., Jiang, C., Wang, X. M., & Huang, L. Q. (2014). Exploiting genes and functional diversity of chlorogenic acid and luteolin biosyntheses in *Lonicera japonica* and their substitutes. *Gene, 534*(2), 408–416.

Zhang, N., Ramachandran, P., Wen, J., Duke, J. A., Metzman, H., McLaughlin, W., et al. (2017). Development of a reference standard library of chloroplast genome sequences, GenomeTrakrCP. *Planta Medica, 83*(18), 1420–1430.