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DNA barcoding for identification of *Melicope pteleifolia* and its close species based on ITS2 sequences

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Abstract

Melicope pteleifolia (*Evodia lepta*), named *San-cha-ku* in traditional Chinese medicine, is commonly used for the treatment of cold and gastropathy. Due to its wide regional distribution and a broad range of closely-related species, *M. pteleifolia* and its close species have been used in different regions of China. However, it is challenging to identify *M. pteleifolia* from other close species by only using traditional methods. Here, we report a molecular identification method based on ITS2 sequences of DNA barcoding to differentiate *M. pteleifolia* from its adulterants or related species. Our results showed that the ITS2 sequence length of *M. pteleifolia* was 221 bp. The K2P model that assesses the relative variation of the ITS2 regions was used to evaluate the genetic distance of *M. pteleifolia* from its closely related species. The inter-specifi distance was 0.1187, which is larger than the intra-specifi distance of 0.0116. The identification efficiency of ITS2 was 100% when evaluated with Blastn. The identification of NJ trees showed that *M. pteleifolia* formed into one clade, which can be distinguished successfully from its adulterants or related species. Our results suggested that *M. pteleifolia* can be identified stably and accurately through evaluating the ITS2 regions.

Keywords: *Melicope pteleifolia*; DNA Barcoding; Identification; ITS2.
Abbreviations: HMMer_hidden Markov model; ITS_internal transcribed spacer; ITS2_internal transcribed spacer 2; K2P_kimura 2-parameter; NJ_neighbor-joining.

Introduction

Melicope pteleifolia (Champ ex Benth) T.G. Hartley, belonging to the family of Rutaceae, is a deciduous shrub or arbor widely distributed in southern China and Southeast Asia (Editorial Committee of Flora Republicae Popularis Sinicae, 1997). *M. pteleifolia* is a medicinal herb and an edible plant, which is used as an antipyretic, anti-inflammatory, and analgesic agent to treat the cold and influenza, epidemic cerebrospinal meningitis (ECM), tonsillitis and other conditions (Liang and Guo, 2009; Yoon et al., 2013; Editorial Committee of Chinese Pharmacopoeia, 1977). Chemical studies have shown that alkaloids, flavonoids, volatile oils, glycosides, et al had been isolated from *M. pteleifolia* (Liang and Guo, 2009; Zhang et al., 2012; Wei et al., 2013; Sichaem et al., 2014). In recent years, *M. pteleifolia* has been used as a major material in Chinese patent medicine. For example, its fresh leaves are used as one of the main ingredients in Guangdong Herbal Tea—a popular healthy drink in China, while its roots and barks, known as 'San-cha-ku' in traditional Chinese medicine, are commonly used in Chinese patent medicine such as "999 Weitai"—a famous brand of traditional Chinese medicine formula for the treatment of various gastritis, and "999 Ganmaoling"—a well-known formula for cold (Mao et al., 2004; Dong et al., 2002; Jiang et al., 2009). However, *M. pteleifolia* is frequently used inappropriately with synonyms or homonyms, i.e, different traditional Chinese medicine names are used in error, for the same plant in different regions, or the same traditional Chinese medicine name is used for different plants in many areas. For instance, *M. pteleifolia* is recorded as 'San-ya-ku' in "Ling Nan Cai Yao Lu" (Lingnan Chinese Medicine Library), but it is known as 'Ji-gu-shu' in China's Guangdong province. Meanwhile, regional differences are an important factor affecting quality. Therefore, it is very important to identify measurable differences between *M. pteleifolia* and its close species from different regions so as to ensure the correct plant is selected for medicine. Several methods have been developed to identify this material. For example, Yang et al (Yang et al., 2008) described the material characteristics of tissues and microscopic characteristics of the powder of *M. pteleifolia* by characteristic identifying and micro-identifying methods. The Thin Layer Chromatography (TLC) method has also been employed by Zeng et al. (2014)

Table 1. The Sequence Characteristics of ITS2 of *Melicope pteleifolia* and its related species.

Sequence characteristics	ITS2
Length in *Melicope pteleifolia*(bp)	221
Length in all taxa(bp)	219-222
GC content range (mean) in *M. pteleifolia*/%	59.3-60.6(59.8)
GC content range (mean) in all taxa/%	56.8-60.6(59.2)
Number of variable sites in *M. pteleifolia*	10
Number of variable sites in all taxa	42

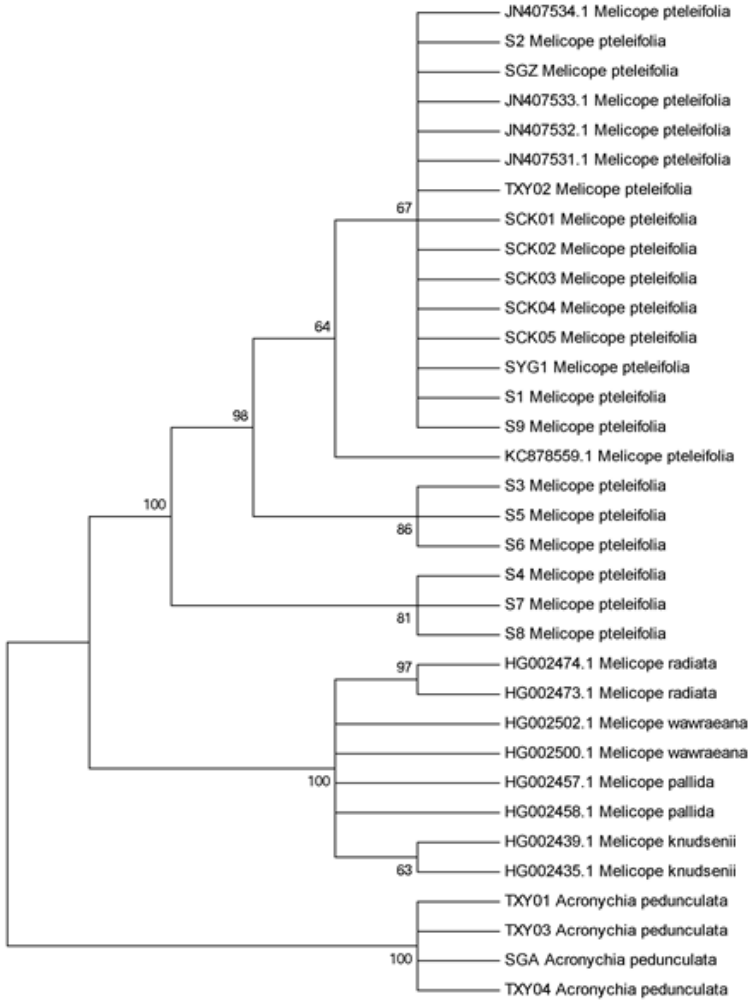

Fig1. NJ tree based on ITS2 sequences from samples.

to identify *M. pteleifolia* material (Zeng et al., 2008). These studies provide some references for the identification of *M. pteleifolia*. However, it is still difficult to identify *M. pteleifolia* based only on its general morphology and microstructure. DNA barcoding can quickly and reliably identify species using a standard genetic sequence that can be amplified by universal primers from a small fragment of the genome. In the last two decades, molecular tools based on DNA sequences of short standardized gene fragments and termed DNA barcodes have been used for species discrimination (Fišer and Buzan EV, 2014). Several DNA regions have been evaluated as DNA barcodes, including *matK*, *rbcL*, *psbA-trnH*, and ITS2, for potential applications (Christina VL and Annamalai A, 2014; Sun et al., 2013; Liu et al., 2012). We established a molecular identification method of DNA barcodes to identify Chinese medicinal materials and found the core barcode ITS2 and *psbA-trnH* to be a complementary locus for plant medica (Chen et al., 2010; Chen et al., 2013). In recent years, this method has been frequently used to identify many kinds of Chinese herbs (Gao et al., 2010; Luo et al., 2012; Chen et al., 2012; Hou et al., 2013). For instance, in the study of Luo (Luo et al., 2010), seven regions (*psbA-trnH*, *matK*, *ycf5*, *rpoC1*, *rbcL*, ITS2, and ITS) identified by DNA barcodes have been tested for their ability to identify 300 samples of 192 species from 72 genera of Rutaceae. Here we identified *M. pteleifolia* and its adulterants or related species through ITS2 sequences. For the first time, we will try to find a more accurate, sensitive, and simple way to distinguish *M. pteleifolia* from other plant material.

Results

Results of DNA extraction and PCR amplification

In this study, the success rates of ITS2 DNA extraction and

Table 2. The intra-specific variable sites in the ITS2 sequence of *M. pteleifolia*.

Site	13bp	25bp	29 bp	34 bp	53 bp	87 bp	108 bp	163 bp	175 bp	220 bp
SGZ	C	T	T	A	A	G	C	A	A	C
JN407534.1					G					
KC878559.1				C						
S3				C			T			T
S4	T	C	G	C		C		T	T	

Table 3. Samples used in this study.

Sample name	Voucher No.	Location	GenBank No.
Melicope pteleifolia	SGZ (powder)	Beijing#	KJ158059
Melicope pteleifolia	TXY02 (stems)	Wuzhou, Guangxi	KJ158060
Melicope pteleifolia	SCK01 (leaves)	Meizhou, Guangdong	KJ158061
Melicope pteleifolia	SCK02 (leaves)	Mashan, Guangxi	KJ158062
Melicope pteleifolia	SCK03 (leaves)	Shizheng, Guangxi	KJ158063
Melicope pteleifolia	SCK04 (leaves)	Tongren, Guangdong	KJ158064
Melicope pteleifolia	SCK05 (leaves)	Pingyuan, Guangdong	KJ158065
Melicope pteleifolia	SYG1 (leaves)	Guangzhou, Guangdong	KJ158066
Melicope pteleifolia	S1 (leaves)	Zhanjiang, Guangdong	KJ158067
Melicope pteleifolia	S2 (leaves)	Shenzhen, Guangdong	KJ158068
Melicope pteleifolia	S3 (leaves)	Wanning, Hainan	KJ158069
Melicope pteleifolia	S4 (leaves)	Yanuoda, Hainan	KJ158070
Melicope pteleifolia	S5 (leaves)	Xinglong, Hainan	KJ158071
Melicope pteleifolia	S6 (leaves)	Xinglong, Hainan	KJ158072
Melicope pteleifolia	S7 (leaves)	Xinglong, Hainan	KJ158073
Melicope pteleifolia	S8 (leaves)	Xinglong, Hainan	KJ158074
Melicope pteleifolia	S9 (leaves)	Bazhaigou, Guangxi	KJ158075
Acronychia pedunculata	SGA (stems)	Anguo, Hebei	KJ158055
Acronychia pedunculata	TXY01 (stems)	Yunnan	KJ158056
Acronychia pedunculata	TXY03 (stems)	Guangzhou, Guangdong	KJ158057
Acronychia pedunculata	TXY04 (stems)	Nanning, Guangxi	KJ158058
Melicope pteleifolia		GenBank	JN407534.1
Melicope pteleifolia		GenBank	JN407533.1
Melicope pteleifolia		GenBank	JN407532.1
Melicope pteleifolia		GenBank	JN407531.1
Melicope pteleifolia		GenBank	KC878559.1
Melicope radiata		GenBank	HG002474.1
Melicope radiata		GenBank	HG002473.1
Melicope pallida		GenBank	HG002457.1
Melicope pallida		GenBank	HG002458.1
Melicope wawraeana		GenBank	HG002502.1
Melicope wawraeana		GenBank	HG002500.1
Melicope knudsenii		GenBank	HG002439.1
Melicope knudsenii		GenBank	HG002435.1

(Standard reference herb, purchased from National Institute of food and drug Control. Batch number 121682-201301)

PCR amplification were 100%. Clear bands were found by DNA gel electrophoresis and high quality sequencing results were obtained from the ITS2 regions.

Analysis of Genetic interspecific divergence and intraspecific variation

The ITS2 sequence lengths of all the *M. pteleifolia* samples were 221 bp and the average content of G+C was 59.8% (Table 1). Ten of the nucleotide variation sites were found (Table 2). The ITS2 sequence was obtained using the HMMer annotation method (http://its2.bioapps.biozentrum.uni-wuerzburg.de/) based on the Hidden-Markov model to remove the conserved 5.8S and 28SrRNA sequences of samples (Hou et al., 2013). The inter-specific distance of *M. pteleifolia* and its related species was 0.1076-0.1400, which is larger than the intra-specific distance (0-0.0371) of *M. pteleifolia*.

The ITS2 sequence of sample SGZ(Table 3): CGCATCATTGCCCCACCCCCACCCTTCTTGGGCAAG GTGGTGTAGGCGGAGAATGGCCTCCCGTGAGCAACC ACCCGCGGTTGGCCGAAAAGTGAGTTCTCGGTGACC AAAGCCGCGATGATCGCTGGTGCAAAATTGCCTCTC GAGTTCACGTCGCGTGCCAACGTCTTCGATAACGGA CTCAAGGACCCTGACGCTCTGCAAAAGCGGAGCTC GCATCG.

Identification of ITS2 using NJ trees and BLAST

The phylogenetic tree was constructed using the NJ method with 1000 bootstrap replicates for the ITS2 sequences (Fig.1). Results showed that *M. pteleifolia* formed into one clade, which can be successfully distinguished from its adulterants (Acronychia pedunculata) or related species (Melicope radiate, Melicope wawraeana, Melicope pallida, Melicope knudsenii). In addition, the results showed that ITS2 regions possess high identification efficiency (100%) when BLAST was applied based on NCBI database (http://www.ncbi.nlm.nih.gov/). In conclusion, through ITS2 sequences, *M. pteleifolia* could be distinguished clearly from its adulterants or related species.

Discussion

To date, due to the high similarities of superficial characters and chemical compositions between closely related species, it is challenging to identify *M. pteleifolia* and its adulterants by characteristic identification and micro-identification methods or the TLC method. However, DNA barcoding has been proposed to be one of the most promising tools for accurate and rapid identification of taxa (Xu et al., 2015). The identification of the ITS2 region in particular has enabled, using the availability of universal primers and high amplification rates, excellent identification ability even in closely related species (Yao et al., 2010). In our PCR reaction, the successful running of 21 samples involving stems and leaves demonstrated that the ITS2 region amplification is simple and reliable. At the same time, the inter-specific K2P distance of *M. pteleifolia* and its related species is obviously larger than the intra-specific K2P distance, and the results of the NJ tree indicated that *M. pteleifolia* and its related species were clustered in a different monophyletic group. From the above analysis that can then be validated by BLAST analysis results, an ITS2 region can accurately distinguish *M. pteleifolia* from its related species. The ITS2 sequence is incapable of differentiating *Melicope wawraeana* et al from the same genus of *Melicope pallida*, with this result suggesting that ITS2 needs to be combined with other barcodes for further distinction. The psbA-trnH, matK, rbcL regions have been amplified in this research as well. Our study showed that the amplification success rate of the matK, rbcL regions was lower than that for ITS2. While the amplification rate of the psbA-trnH region is as high as the ITS2 region, its identification efficiency is clearly lower than ITS2 region. In this study, there were no variation sites of ITS2 sequences between the samples from Guangdong and Guangxi province, but variation sites did existed among samples from the Hainan province (3 or 7, respectively). These experimental results may provide a useful reference for distinguishing among different samples' origins. Some research has evaluated candidate DNA barcodes, like ITS2, ITS, psbA-trnH, matK, rbcL and CO1. Chao et al. tested 4 markers: ITS2, psbA-trnH, matK, and rbcL, to compare these candidate DNA barcodes in their abilities to distinguish Bupleuri radix (Chaihu) from its adulterants. The results showed that ITS2 had the best performance and could serve as a potentially useful barcode for the Bupleurum species, with psbA-trnH as a supplementary locus (Chao et al., 2014). Zheng et al collected 478 sequences from six candidate DNA barcodes (ITS2, ITS, psbA-trnH, rbcL, matK, and COI) from 29 species of Radix Astragali and adulterants, with the results showing that the ITS sequence was the optimal barcode for identifying Radix Astragali and its adulterants (Zheng et al., 2014). Compare the efficiency of PCR amplification and ability to discriminate species to find better DNA barcodes (Yan et al., 2014; Wang et al., 2014). The most common among them were ITS2 and psbA-trnH, which is consistent with our study.

Materials and Methods

Plant materials

Twenty-one *M.pteleifolia* and adulterant samples were collected from different regions of China, and a standard sample was purchased from the National Institute of Food and Drug Control (Beijing, China). The experimental materials have been identified and preserved at Minzu University of China. The rest of the sample sequences were downloaded from GenBank (Table 3). Whole sample sequences appearing in this study have covered all the ITS2 genotypes of *M. pteleifolia* in GenBank. Fresh leaves were dried in silica gel and roots were surface-sterilized with 75% ethanol and dried in silica gel.

DNA extraction

After being disinfected with 75% alcohol, 15 mg of dry leaves or 30 mg of dry stems were grinded for 1 min with a DNA extraction grinder (Restech MM400, Germany). A plant genomic kit (Tiangen Biotech Co., China) was then used according to the instructions to extract total genomic DNA.

PCR amplification and sequencing

ITS2 regions were amplified in a Peltier Thermal Cycler (Bio Red Lab Inc., USA) using a universal primer. Forward primer ITS2F:5′-ATGCGATACTTGGTGTGAAT-3′; Reverse primer ITS3R: 5′-GACGCTTCTCCAGACTACAAT-3′ (Chen, 2012). PCR reaction was performed in 25μl volumes, including 2μl of DNA-template, 12.5 μl of Taq Master mix (Aidlab Biotech Co., China), 1μl of each primer (2.5μM), and 8.5μl of ddH_2O. In the negative control group, the template was replaced by ddH_2O. Reaction procedures were as follows: 94 ºC 5 min; 40 cycles (94 ºC 30 s, 56 ºC 30 s, 72 ºC 45 s); 72 ºC 10 min (Han et al., 2011).
Purified PCR products were bidirectionally sequenced by the sequencing center at Molecular Biology and Chemical (Mbchem, Shanghai).

Data analysis

After sequencing, forward and reverse trace files were trimmed and assembled using the CodonCode Aligner V 3.7.1 (CodonCode Co., USA). Genetic distances were then calculated by MEGA 5.0 according to the Kimura 2-Parameter (K2P) model, and neighbor-joining (NJ) trees were constructed to identify *M. pteleifolia* and its related species (Xin et al., 2012; Pang et al., 2011). In addition, the BLAST method was used to identify *M. pteleifolia* and its related species with the Species Identification System (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Conclusion

In summary, ITS2 sequence identification was found to be an efficient method for the rapid and accurate identification of *M. pteleifolia*. The intraspecific variation in selected regions was low. Through a comparison of intragenomic and intraspecific variation, we concluded that the ITS2 sequence can be used to distinguish *M. pteleifolia* from its adulterants or related species.

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References

Chen SL, Yao H, Han JP, Liu C, Song JY, Shi LC, Zhu YJ, Ma XY, Gao T, Pang XH, Luo K, Li Y, Leon C (2010) Validation of the ITS2 region as a novel DNA barcode for

identifying medicinal plant species. PLoS One. 5: e8613
Chen SL, Yao H, Han JP, Xin TY, Pang XH, Shi LC, Luo K, Song JY, Hou DY, Shi SM, Qian ZZ (2013) Principles for molecular identification of traditional Chinese materia medica using DNA barcoding. Zhongguo Zhong Yao Za Zhi 38: 141-148.
Chen SL (2012) Molecular Identification of Chinese Materia Medica Using DNA Barcodes; People ' s Medical Publishing House, Beijing, China.
Chao Z, Zeng W, Liao J, Liu L, Liang Z, Li X (2014) DNA barcoding Chinese medicinal Bupleurum. Phytomedicine. 21: 1767-73.
Chen Q, Liu Y, Liu Z, Chen SL, Chen KL (2012) DNA bacording of Verbenaceae medicinal plant by using ITS2 and psbA-trnH region. Zhongguo Zhong Yao Za Zhi. 37: 1107-1113.
Christina VL, Annamalai A (2014) Nucleotide based validation of Ocimum species by evaluating three candidate barcodes of the chloroplast region. Mol Ecol Resour. 14: 60-8.
Dong XH, Hu FL, Li SR, Yang ZX, Ye JX, Wang SX, Zhang P (2002) Efficacies of Sanjiuweitai-based quadruple regimens for the treatment of peptic ulcer disease and eradication of Helicobacter pylori. China J New Drugs. 11: 477-9.
Editorial Committee of Flora Republicae Popularis Sinicae (1997) Flora Republicae Popularis Sinicae; Science Press: Beijing, China.
Editorial Committee of Chinese Pharmacopoeia (1977) Chinese Pharmacopoeia; People ' s Medical Publishing House: Beijing, China.
Fiser PZ, Buzan EV (2014) 20 years since the introduction of DNA barcoding: from theory to application. J Appl Genet. 55: 43-52.
Gao T, Yao H, Song JY, Liu C, Zhu YJ, Ma XY, Pang XH, Xu H, Chen SL (2010) Identification of medicinal plants in the family Fabaceae using a potential DNA barcode ITS2. J Ethnopharmacol. 130: 116-21.
Han JP, Li MN, Luo K, Liu MZ, Chen XC, Chen SL (2011) Identification of Daturae flos and its adulterants based on DNA barcoding technique. Yao Xue Xue Bao. 46: 1408-12.
Hou DY, Song JY, Shi LC, Ma XC, Xin TY, Han JP, Xiao W, Sun ZY, Cheng RY ,Yao H (2013) Stability and accuracy assessment of identification of traditional Chinese materia medica using DNA barcoding: a case study on Flos Lonicerae Japonicae. Biomed Res Int. 2013: 549073.
Jiang J, Pan JY, Yang HM, Qiu YS (2009) Experimental study of long-term toxicity of Sanjiu weitai granules in rats. Chin J Clin Pharmacol. 25: 249-51.
Liang Y, Guo LB (2009) Research Progresses on Chemical Compositions and Pharmacological Activities of Evodia lepta (Spreng.) Merr. Modem Chinese Medicine. 11: 14-15.
Liu Z, Zeng X, Yang D, Chu G, Yuan Z, Chen S (2012) Applying DNA barcodes for identification of plant species in the family Araliaceae. Gene. 499: 76-80.
Luo K, Ma P, Yao H, Xin TY, Hu Y, Zheng SH, Huang LF, Song JY (2012) Identification of gentianae macrophyllae radix using the ITS2 barcodes. Yao Xue Xue Bao. 47: 710-7.
Luo K, Chen S, Chen K, Song J, Yao H, Ma X, Zhu Y, Pang X, Yu H, Li X, Liu Z (2010) Assessment of candidate plant DNA barcodes using the Rutaceae family. Sci China Life Sci. 53: 701-708.
Mao YQ, Mou ZX, Zhang YF, Zhang YL, Ruan Y, Gao HF, Xie WN, Lu YC, Tan HR (2004) Experimental study on the pharmacology of 999 ganmaoling, a compound recipe of Chinese and Western materia medica. Chin J Integrated Trad Western Medicine. 24: 726-30.
Sichaem J, Jirasirichote A, Sapasuntikul K, Khumkratok S, Sawasdee P, Do TM,Tip-Pyang S (2014) New furoquinoline alkaloids from the leaves of Evodia lepta. Fitoterapia. 92: 270-3.
Pang X, Song J, Zhu YJ (2011) Applying plant DNA barcodes for Rosaceae species identification. Cladistics. 27: 165−170.
Sun XQ, Bai MM, Yao H, Guo JL, Li MM, Hang YY (2013) DNA barcoding of populations of Fallopia multiflora, an indigenous herb in China. Genet Mol Res. 12: 4078-89.
Wang ZT, Hao H, Lin LZ, Yu Y, Li SY (2014) Identification of Smilax glabra and its related species based on psbA-trnH sequence. Zhong Yao Cai. 37: 1368-71.
Wei HL, Zhou SX, Jiang Y, Song YL, Li J, Tu PF (2013) Chemical constituents from leaves of Evodia lepta. Zhongguo Zhong Yao Za Zhi. 38: 1193-7.
Xin TY, Yao H, Luo K, Xiang L, Ma XC, Han JP, Lin YL, Song JY, Chen SL (2012) Stability and accuracy of the identification of Notopterygii Rhizoma et Radix using the ITS/ITS2 barcodes. Yao Xue Xue Bao. 47: 1098-105.
Xu S, Li D, Li J, Xiang X, Jin W, Huang W, Jin X, Huang L (2015) Evaluation of the DNA barcodes in Dendrobium (Orchidaceae) from mainland Asia. PLoS One. 10: e0115168.
Yang WL, Mao CN, Liu MS, Zhang JQ (2008) Pharmacognocy study on Lignum et folium Evodia Lepta. China Tropical Medicine. 8:851-853.
Yan L, Liu J, Möller M, Zhang L, Zhang X, Li D, Gao L (2014) DNA barcoding of Rhododendron (Ericaceae), the largest Chinese plant genus in biodiversity hotspots of the Himalaya-Hengduan Mountains. Mol Ecol Resour. doi: 10.1111/1755-0998.12353.
Yao H, Song J, Liu C, Luo K, Han J, Li Y,Pang X, Xu H, Zhu Y, Xiao P,Chen S (2010) Use of ITS2 region as the universal DNA barcode for plants and animals. PLoS One. 5: 13102.
Yoon JY, Jeong HY, Kim SH, Kim HG, Nam G, Kim JP, Yoon DH, Hwang H, Kimc TW, Hong S, Cho JY (2013) Methanol extract of Evodia lepta displays Syk/Src-targeted anti-inflammatory activity. J Ethnopharmacol. 148(3):999-1007.
Zheng S, Liu D, Ren W, Fu J, Huang L, Chen S (2014) Integrated analysis for identifying radix astragali and its adulterants based on DNA barcoding. Evid Based Complement Alternat Med. 2014: 843923.
Zhang Y, Yang LJ, Jiang K, Tan CH, Tan JJ, Yang PM, Zhu DY (2012) Glycosidic constituents from the roots and rhizomes of Melicope pteleifolia. Carbohydr Res. 361: 114-9.
Zeng CY, Gao YJ, Zhong XW (2008) Studies on TLC of Yuekang Waigan Bagged Tea. *Chinese Archives of* Traditional Chinese Medicine. 26:2247-2248.