Genome-Based Approaches to the Authentication of Medicinal Plants

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- Medicinal plants
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Abstract

Medicinal plants are the source of a large number of essential drugs in Western medicine and are the basis of herbal medicine, which is not only the primary source of health care for most of the world's population living in developing countries but also enjoys growing popularity in developed countries. The increased demand for botanical products is met by an expanding industry and accompanied by calls for assurance of quality, efficacy and safety. Plants used as drugs, dietary supplements and herbal medicines are identified at the species level. Unequivocal identification is a critical step at the beginning of an extensive process of quality assurance and is of importance for the characterization of the genetic diversity, phylogeny and phylogeography as well as the protection of endangered species. DNA-based methods have been developed for the identification of medicinal plants. Nuclear and chloroplast DNA is amplified by the polymerase chain reaction and the reaction products are analyzed by gel electrophoresis, sequencing, or hybridization with species-specific probes. Genomic fingerprinting can differentiate between individuals, species and populations and is useful for the detection of the homogeneity of the samples and presence of adulterants. Although sequences from single chloroplast or nuclear genes have been useful for differentiation of species, phylogenetic studies often require consideration of DNA sequence data from more than one gene or genomic region. Phytochemical and genetic data are correlated but only the latter normally allow for differentiation at the species level. The generation of molecular "barcodes" of medicinal plants will be worth the concerted effort of the medicinal plant research community and contribute to the ongoing effort of defining barcodes for every species on earth.

Introduction

Plants have been used for medicinal purposes not only by humans since prehistoric times [1], [2] but are also used to treat various ailments by our closest relatives, the African great apes [3], [4]. To date, medicinal plants are the source of a large number of chemical compounds used as drugs in Western medicine and serve as the primary therapeutic resource for most of the world's population living in developing countries [5], [6], [7], [8], [9]. At the same time the use of herbal preparations for health care purposes is gaining popularity in developed countries [10], [11]. The increased demand for botanical products is met by an expanding industry and accompanied by calls for assurance of quality, efficacy and safety [12], [13].

The botanical sources of herbal supplements and medicines are identified at the species level by their Latin scientific names and the plant species is the basic unit for the preparation of herbal formulations. National pharmacopoeias such as that of China [14] as well as recent drug monographs (e.g., ref. [15]) prepared for the botanical industry and regulators always start their description of herbal drugs by naming the botanical species used for its preparation. Unequivocal identification and authentication of the plants used for production is therefore an elementary and critical step at the beginning of an extensive quality assurance process. Unfortunately, substitution or adulteration either intentionally, e.g., motivated by the desire to maximize financial gains, or unintentionally, e.g., by clerical errors or lack of knowledge, are not rare occurrences [16] and can have tragic consequences [17]. Authentica-

tion is also of importance for the characterization of the genetic diversity [18], [19], phylogeny and phylogeography [20], [21] as well as the protection and management of endangered species [22].

Identification of plants at the species level is traditionally achieved by careful examination of the specimen's macroscopic and microscopic morphology. This work usually needs to be performed by a specially trained expert. However, morphological identification is often not possible when the original plant material has been processed. Therefore, additional methods of identification at the species level have been sought and genome-based methods have been developed for the identification of medicinal plants starting in the early 1990 s [17], [23]. This work followed in the footsteps of the use of DNA for plant systematics in the preceding two decades [24], [25] and was greatly facilitated by the invention of the polymerase chain reaction (PCR) and the introduction of a heat-stable DNA polymerase from the thermophilic bacterium *Thermus aquaticus* [26]. Together, these two achievements have revolutionized the way scientists work with DNA and made molecular cloning and DNA-based analysis accessible to workers in virtually every field concerned with living matter. In fact, molecular taxonomists now envision cataloging all living species on earth using so-called DNA barcodes, the nucleotide sequence of a short DNA fragment [27], [28], [29].

Here, we review the published work using genome-based approaches to the authentication of medicinal plants. Much of this work specifically relates to the authentication of plants used as sources of drugs in Chinese medicine. Chinese herbal medicine is part of a system of medical thought and practice that is distinctly different from that of Western medicine [30] and is the most widely practiced form of herbalism worldwide. In recent years, a number of factors have stimulated interest in Chinese medicine in the West, where an increasing number of patients and medical practitioners use herbal medicines as a supplement to or substitute for prescription drugs. Therefore, interactions between herbal and Western medicines have become an important issue in clinical practice [31], [32]. In China and Japan herbal medicines are listed in the national pharmacopoeias and their use is recognized and promoted by official health care policy on equal footing with Western style (single chemical entity) prescription drugs [33], [34], [35], [36].

Molecular Biological Techniques used for Genome-Based Authentication

An overview and description of the various techniques that have been used for genome-based authentication of medicinal plants is presented in ◉ **Table 1**. These procedures can be broadly divided into two general approaches. In one approach, investigators determine the nucleotide sequence of one or more genetic loci ("genes") in the plants of interest and identify nucleotide sequences that are characteristic (i.e., inherited by all members) of a given species. Examples of techniques that are based on this approach and are described in ◉ **Table 2** include allele-specific diagnostic PCR, amplified refractory mutation system (ARMS) and multiplex amplification refractory mutation system (MARMS), DNA microarray, and DNA sequencing. In a second approach, rather than focusing on specific genetic loci, researchers make use of species-specific variations (polymorphisms) of the nucleotide sequence that are spread randomly over the entire genome resulting in characteristic "fingerprints" of genomic DNA. Examples of techniques that are based on this approach and are described in ◉ **Table 2** include amplified fragmented length polymorphism (AFLP), arbitrarily primed PCR (AP-PCR), direct amplification of length polymorphism (DALP), randomly amplified polymorphic DNA (RAPD), restriction length polymorphism (RFLP), inter simple sequence repeat anchored PCR and simple sequence repeat polymorphism (SSR). The PCR and its numerous variations are central to both approaches and virtually all of the published genome-based authentication work employs this technique.

PCR was originally developed for the directed amplification of predetermined regions of genomic DNA using primers with a specific sequence and is used in this way for the cloning and sequencing of specific genetic loci. However, PCR can also be used for the amplification of random stretches of DNA using primer pairs with arbitrary nucleotide sequences [37]. With arbitrary primers, the PCR yields a mixture of amplified products (amplicons) of various sizes that can be analyzed by gel electrophoresis. The amplicon patterns reflect the polymorphisms in different genomic DNA samples and are termed RAPD. This version of the PCR is a more rapid and less laborious replacement for the digestion of genomic DNA by restriction enzymes for the characterization of RFLP [38]. Both RAPD and RFLP result in a mixture of DNA fragments. The fragments are sorted by size using gel electrophoresis. The DNA is visualized either directly in the gel using fluorescent dyes (e.g., ethidium bromide) or indirectly using radioactively labeled probes, which are hybridized to the DNA following its transfer ("blotting") from the gel to a solid membrane (e.g., nitrocellulose or nylon). The latter procedure is referred to as Southern blotting using the name of its inventor as an eponym. The pattern obtained with a specific DNA sample is termed its "fingerprint". Once a "fingerprint" has been established for a control sample, the appearance of additional amplicons in test samples signals the presence of impurities or unexpected genetic variation. RAPD was used by some of the early workers using genome-based methods for the authentication of medicinal plants and their RAPD protocols as well as other modified versions of PCR have been collected in a recently published booklet [39]. As a PCR-based procedure, RAPD requires only nanogram amounts of genomic DNA and rapidly and efficiently generates a large number of genomic markers. Although RAPD is suitable for both the rapid sample authentication as well as the assessment of sample purity, it is often not easy to replicate fingerprint patterns established in one laboratory in another because even slight (instrumentation-dependent) variations during the PCR can result in variant fingerprints even when samples of the same genomic DNA are used. In contrast, sequencing will always yield the same result independent of the particular instrumentation used. DNA sequence data can be deposited as simple text strings (with explanatory meta data) in electronic databases such as GenBank and mined easily using text-based bioinformatics tools in contrast to gel-based fingerprints, which will require more complicated image analysis software. Finally, the advent of automated DNA sequencers and DNA microarrays has resulted in a considerable drop in the costs of using these techniques and should favor their more general and widespread use for genome-based authentication of medicinal plants.

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Table 1 Molecular biological methods used for the authentication of medicinal plants

Name	Acronym	Explanation
Polymerase chain reaction	PCR	PCR provides an *in vitro* method for the rapid enzymatic amplification of fragments of deoxyribonucleic acid (DNA) [114], [115]. In the PCR procedure, two oligonucleotide primers (often referred to as "upstream" and "downstream" or "forward" and "reverse" primers) that are complementary to the 5′ and 3′ flanking sequences of the DNA to be amplified are used to prime a heat-stable DNA polymerase that performs the copying of each strand of DNA. The denaturation of the DNA double helix, the annealing of the oligonucleotide primers to each complementary strand, and the synthesis of new strands by DNA polymerase are performed at their optimal temperature resulting in a three-step reaction. PCR is conducted in fully programmable thermocyclers that change the reaction temperatures at each step automatically [116].
Allele-specific diagnostic PCR		Primers with allele specific 3′ ends and labeled with different fluorochromes at their 5′ end are used together with a common primer in PCR [117]. The resulting amplicons can be analyzed by gel electrophoresis or capillary electrophoresis using an automated DNA sequencer.
Amplification refractory mutation system	ARMS	This variation of the PCR is based on the fact that the primers only bind to their target sequence when their 3′-ends are complementary. Oligonucleotides with mismatched ("mutated") 3′ end residues will not bind to the "normal" target sequence and no amplification will take place [118].
Amplified fragmented length polymorphism	AFLP	In this technique, genomic DNA is digested with restriction enzymes. In a ligation reaction specific oligonucleotide adapters are added to the ends of the fragments, which can then be selectively amplified by PCR using primers that are complementary to the adapter and restriction site sequence [119].
Arbitrarily primed PCR	AP-PCR	Similar to RAPD but PCR is performed using sets of two longer primers (>18 nucleotides) of arbitrary sequence.
Direct amplification of length polymorphism	DALP	PCR is conducted with variable forward primers that contain a universal core sequence at their 5′ end and a constant reverse primer resulting in multiple amplicons that can be separated by gel electrophoresis, isolated and directly sequenced [120].
Multiplex PCR		PCR with multiple sets of forward and reverse primers in the same reaction resulting in parallel amplification [116].
PCR-selective restriction	PCR-SR	PCR amplicons obtained with gene specific primers are cut with restriction enzymes and analyzed by gel electrophoresis [121].
Randomly amplified polymorphic DNA	RAPD	Genomic DNA (gDNA) is amplified by PCR using a single, short (10 nucleotides) primer with arbitrary sequence resulting in multiple amplicons of different lengths ("fingerprint" pattern) that are analyzed by gel electrophoresis [37].
Sequence characterized amplified region	SCAR	Distinct amplicons obtained by RAPD are sequenced and amplicon specific primers are designed for use in PCR [122].
Restriction length polymorphism	RFLP	Genomic DNA is cut with sequence specific DNA restriction endonucleases resulting in the generation of a number of small fragments of various lengths, which are separated according to their molecular size by gel electrophoresis. The band pattern obtained with a specific DNA source and a specific restriction enzyme is called a DNA fingerprint of that source.
DNA microarray		A DNA microarrays, also often referred to as gene chip, DNA chip, or gene array, consists of a solid support matrix (e. g. a glass slide, silicon chip or synthetic membrane) to which DNA has been covalently bound in the form of a collection of microscopic spots [123]. Each spot contains DNA of a defined sequence that is referred to as the probe. Fluorescently labeled target DNA is hybridized to the chip, which is washed and then analyzed using a microarray reader.
DNA sequencing		DNA sequencing is now almost exclusively performed using cycle sequencing, which is conducted using a heat stable DNA polymerase and fluorescently labeled dideoxynucleotides in a thermocyler. The resulting polymerase products are separated according to length using capillary electrophoresis, detected by laser-induced fluorescence and automatically analyzed by computer software [124]. Older methods making use of radioactively labeled nucleotides and gel electrophoresis are still in use and may be the only option, when access to automated sequencers is not available.
Inter simple sequence repeat-anchored PCR	ISSR-PCR	In ISSR-PCR, primers anchored at simple sequence repeat (SSR) sequences (e. g., CACACACA; see below) are used to amplify the DNA regions between the flanking SSR [125].
Multiplex amplification refractory mutation system	MARMS	Multiplex PCR using a common primer and multiple mutation specific primers as used in ARMS [126].
Simple sequence repeat polymorphism	SSR	Simple sequence repeats (SSRs) or microsatellites are short sequence motifs consisting of 2 or more nucleotides (e. g., CA and ATG), which repeat in tandem (e. g., CACACA and ATGATGATG). The repeats vary in length (e. g., CACACA vs. CACACACACACA) and are ubiquitously and randomly distributed in all eukaryotic genomes. The length-polymorphisms can be easily detected by gel electrophoresis of amplicons generated by PCR using unique pairs of primers flanking the repeat [127].

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Table 2 Nuclear and chloroplast genes used for authentication of medicinal plants

Gene	Genome	Explanantion
18S rRNA	Nuclear	The 18S ribosomal ribonucleic acid (rRNA) sequences have been widely used for phylogenetic studies in plants [128].
Internal transcribed spacers (ITS) of 18S, 5.8S and 26S rRNA	Nuclear	In land plants, the 18S, 5.8S and 26S rRNA genes form a linearly arrayed unit (a cistron) in which the individual coding regions separated by 2 internal transcribed spacers (ITS; ITS1 between the 18S and 5.8S genes and ITS2 between 5.8S and 26S genes). The cistron itself is tandemly arrayed separated by external transcribed spacers (ETS) on one or more chromosomes [57], [60], [129]. The ITS region has been used in many phylogenetic studies [58].
Intergenic spacer of the 5S rRNA (5S gene spacer)	Nuclear	In land plants, the genes for the 5S ribosomal RNA (rRNA) are arrayed as tandem repeats separated by intergenic spacers on one or more chromosomes [57]. The 5S rRNA sequence has been used for construction of the phylogenetic tree of major organisms [61].
26S rRNA	Nuclear	The entire coding region of the 26S rRNA gene can be amplified by DNA and was reported to provide ~3 times more phylogenetically informative characters than the 18S rRNA. The 26S rRNA sequence consists of conserved core and highly variable expansion regions [128].
atpA, atpB, atpF, atpH	Chloroplast	Single copy chloroplast genes coding for the ATP synthase subunits α (atpA), β (atpB), I (atpF), and δ (atpH)), res [130].
chlB	Chloroplast	A chloroplast gene coding for a subunit of the light-independent protochlorophyllide reductase that catalyzes the reduction of protochlorophyllide to chlorophyllide in photosynthetic bacteria, algae, and gymnosperms but is not present in angiosperms [131].
matK	Chloroplast	The matK gene, which is located within the trnK intron and comprises ~1.6 kbp. It is assumed to be involved in the splicing of group II introns [132].
psbA, psbK, psbI	Chloroplast	The pbs genes code for proteins of photosystem II.
rbcL	Chloroplast	Large subunit of the enzyme ribulose-1,5-biphosphate carboxylase (rbcL) is one of the largest (~1.4 kbp) genes in the chloroplast genome. It has been sequenced in a large number of plants beginning in the mid-1980s [55], [56].
rp14, rpl16	Chloroplast	Chloroplast genes coding for the ribosomal proteins L14 and L16, constituents of the large subunit (50S) of the chloroplast ribosome. The chloroplast (70S) and nuclear (80S) ribosomes are of different size [130].
rpoB, rpoC1	Chloroplast	Chloroplast gene coding for DNA-directed RNA polymerase beta and gamma chains, respectively.
rps16	Chloroplast	Chloroplast gene coding for the ribosomal proteins S16, a constituent of the small subunit of the chloroplast ribosome.
trnC, trnD, trnF, trnK, trnL	Chloroplast	Genes coding for the transfer RNA (tRNA) for cystein, aspartate, phenylalanine, lysine, and leucine, respectively. Chloroplast genomes code for 20 to 40 different tRNAs [130]. Regions used in molecular taxonomy include the trnL intron and various tRNA intergenic spacer regions [133].

Microchip-Based Authentication of Medicinal Plants

The desire to speed up the often slow and labor-intensive molecular analyses and reduce costs, has driven research and engineering efforts aimed at the automation and miniaturization of molecular biological analytical techniques and the development of miniature chip-based analytical devices with the goal to build a "lab-on-a-chip" [40], [41], [42], [43]. Our own work in this regard has been aimed at the development of microchip-based devices integrating sample preparation, amplification, detection, and analysis for the DNA-based identification of traditional Chinese herbal materials [44], [45], [46], [47], [48]. We chose silicon as primary and glass as secondary substrates for the fabrication of these devices. Silicon, the paramount substrate for the fabrication of electronic microchips, also offers a number of important advantages for the fabrication of lab-on-a-chip devices and we have recently shown that commonly used microfabrication techniques used in the production of electronic circuits can be modified to include biological materials such as DNA and even protein [49]. Using microfabrication methods, we built silicon-based microchips integrating PCR reactors with built-in electrochemical detection or DNA microarrays and demonstrated their use for the genotyping of Chinese medicinal plants [46], [47]. This work demonstrated that the chips are suitable for the use in the design of automated systems for industrial use and even battery-operated, hand-held devices used as mobile instrumentation in the field.

Molecular Basis of Genome-Based Authentication

Plant DNA comprises three independently replicated genomes. In addition to the nuclear genome that is organized in chromosomes, plants contain circular chloroplast and mitochondrial genomes. The nuclear DNA content (C-value) varies approximately 1000-fold across the angiosperms but exact C-values based on genome sequencing have not been obtained for any angiosperm to date [50]. The chloroplast genome in angiosperms ranges in size between 120 and 220 kb [51] and the plant mitochondrial genome varies in size from 200 kb in *Brassica* to over 2.5 Mb in watermelon and is substantially larger than that in animals,

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which is only between 15 – 18 kb [52]. Interestingly, "whole" genome size determined by sequencing is generally smaller than the C-values indicate, as considerable amounts of genomic DNA cannot be cloned and sequenced with currently available techniques [50]. For example, the *Arabidopsis* Genome Initiative estimated the "genome" size of *Arabidopsis thaliana* at ~125 Mb (115.4 Mb in the sequenced regions plus an estimated 10 Mb in unsequenced regions) but recent data indicate that it may be considerably larger at 157 Mb [50].

The use of genome-based methods for the authentication of medicinal plants should be seen in the context of plant phylogenetic studies and a general effort aimed at barcoding of all plants [53], [54], [55], [56], [57]. Genetic loci commonly used for the authentication of medicinal plants have included the internal transcribed spacers (ITS) that separate the coding regions of the nuclear 5.8S, 18S and 26S rRNA genes [58], [59], [60] and the intergenic spacers that separate multiple repeated copies of the nuclear 5S rRNA gene [61]. On the other hand, genetic loci used in phylogenetic studies include several chloroplast-based genes [55], [56] such as atpF, matK, rbcL, rpoB, and rpoC1, the trnL intron and intergenic spacers between the trnC-trnD, trnL-trnF, trnH-psbA, and psbK-psbKI genes. It is noteworthy that the ITS and 5S spacers have been found to lack sufficient discriminatory power in some phylogenetic studies. In fact, sequence data from a single gene have proved to be insufficient for barcoding purposes in plants because multiple closely related species have been found to possess identical sequences at some loci. Consequently, the consensus view has developed that the unequivocal identification and barcoding of all plant species will require consideration of sequence data from more than one locus [53], [54], [62]. The generation of molecular "barcodes" of medicinal plants and deposition of sequence data in publicly accessible databases will be worth the concerted effort of the medicinal plant research community and contribute to the ongoing effort of defining barcodes for every (plant) species on earth. Along these lines, future studies aimed at the authentication of medicinal plants using genomic methods should focus on genetic loci that have been found useful for barcoding of plants in general in addition to those previously described in the literature.

Application of Genome-Based Authentication

An overview of work that has been performed for the genome-based authentication of medicinal plants is presented in **Table 3**, which collates information from 82 published papers. The columns of the Table contain (from left to right): 1) an alphabetical list of the scientific names of the medicinal plant species that have been investigated (Plant) with information on 2) the plant parts (e.g., leave or root; Part) used for DNA extraction and 3) their condition (e.g., fresh or dry; Condition), an indication of whether 4) a voucher specimen was retained (Voucher), 5) the method (e.g., DNA sequencing; Method), 6) the genetic loci used (Gene) and 7) the number corresponding to the original paper in the list of references (Ref).

Species that have been investigated using genome-based methods for authentication include plants of economical importance such as *Panax* [17], [63], [64], [65], [66], [67], [68], [69], [70], [71], [72], [73], [74], [75], *Fritillaria* [76], [77], [78], [79], [80], and *Ephedra* [81], [82], [83], [84], [85]. Published work furthermore includes species of forensic importance such as *Cannabis* [86], [87], [88], species threatened by extinction such as the wild orchid *Dendrobium* [89], [90], [91], [92], [93], [94], [95], [96], [97], [98], [99], [100], [101], species of unclear phylogenetic relationship such as *Astragalus* [20], [102], [103], [104], [105], [106], and various toxic species such as *Aconitum*, *Datura* and *Strychnos* [44]. The data show that DNA was generally isolated from fresh leaves, stems or roots but in some cases also from dried material, crude drug, extracts and even finished products such as herbal teas, tablets and capsules [85]. Most of the studies included morphological identification of the plants by experts and deposition of voucher specimens in herbaria and museums. Availability of voucher specimens is useful in case potential discrepancies between past and future studies need to be resolved.

A large number of studies have used PCR to establish genetic markers for the authentication of medicinal plants and detection of adulterants. The PCR is one of the most sensitive analytical techniques available and using carefully optimized conditions, it can be used to detect the presence of a single template molecule. In practice, however, pushing the limit of detection is prone to contamination artifacts. Therefore, it is better to use sufficient amounts of good quality template DNA that is free of PCR-inhibiting contaminants than to carry out PCR with a high number of amplification cycles (>35). The best method for the extraction and purification of DNA from a particular plant or drug sample needs to be established empirically. Techen and colleagues [85] showed that the success of PCR was dependent on both the type of source material (raw plants, herbal teas, tablets, capsules) as well as the specific brand of commercial DNA extraction kit used. Following optimization of extraction and PCR, these workers reported correct identification of *Ephedra* species in complex herbal mixtures containing as little as 1 : 1000 part Ephedra tissue [85].

Several investigations examined the correlation of genetic markers with intra- and interspecies geographical and phytochemical variation. For example, workers using the DNA sequence of the 5S rRNA intergenic spacer domain as species identifier found both intra- and interspecies differences in the phytochemical fingerprints established by HPLC [105], [107], [108]. However, only DNA data could resolve species level differences in *Rehmannia* [18]. Not surprisingly, whole-genome RAPD or AP-PCR patterns exhibited more variation at the species level than the sequences of single DNA regions. For example, samples of *Astragalus membranaceus* collected from different geographical regions in China exhibited identical ITS1 sequences but different AP-PCR fingerprints [105]. Similarly, AP-PCR or RAPD fingerprints differentiated samples of *Codonopsis pilosula* from different regions in China [109]. Fruits from *Vitex rotundifolia* obtained from 14 different locations in China could be divided into four closely matching groups based on chemical fingerprinting using HPLC and DNA fingerprinting based on inter simple sequence repeat (ISSR)-anchored PCR [19]. Roots of *Panax notoginseng* collected from a single farm exhibited variation in their AFLP fingerprints which correlated with morphological differences such as variations in leaf color and phytochemical differences such as saponin content [67]. On the other hand, a study of cultivated *Ephedra* plants from different regions in China revealed not only the presence of both *Ephedra sinica* and *Ephedra intermedia* in the same field but also the occurrence of plants with markers for either species and varied morphology [83]. Dong and colleagues determined the DNA sequences of the 5S rRNA spacer, ITS and the 18S rRNA coding region in 10 different taxa of *Astragalus* and used several different bioinformatics tools to construct phylogenetic trees with each genetic region

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Table 3 Quick reference to publications on the application of genome-based methods for the authentication of medicinal plants sorted by species and quick references to experimental methods used (blank = no information provided)

Plant (scientific name)	Part	Condition	Voucher	Method	Gene	Ref
Aconitum carmichaeli	Leaves	Fresh	Yes	PCR, sequencing; microarray (silicon)	5S gene spacer	[44]
Aconitum napellus	Leaves		Yes	AFLP	N/A (not applicable)	[134]
Aconitum pendulum				PCR, sequencing; microarray (silicon)	trnL	[44]
Actaea racemosa	Leaves		Yes	AFLP	N/A	[134]
Actaea cordifolia	Leaves		Yes	AFLP	N/A	[134]
Actaea podocarpa	Leaves		Yes	AFLP	N/A	[134]
Actaea pachypoda	Leaves		Yes	AFLP	N/A	[134]
Adenophora hunanensis		Fresh, crude drug	Yes	PCR, sequencing	5S gene spacer	[135]
Adenophora stricta		Fresh, crude drug	Yes	PCR, sequencing	5S gene spacer	[135]
Adenophora tetraphylla		Fresh, crude drug	Yes	PCR, sequencing	5S gene spacer	[135]
Agastache foeniculum			Yes	PCR, sequencing	18S rRNA; matK	[136]
Agastache rugosa			Yes	PCR, sequencing	18S rRNA; matK	[136]
Alisma canaliculatum	Rhizome	Dried	Yes	PCR, sequencing; RFLP; ARMS	ITS	[137]
Alisma gramineum	Rhizome	Dried	Yes	PCR, sequencing; RFLP; ARMS	ITS	[137]
Alisma lanceolatum	Rhizome	Dried	Yes	PCR, sequencing; RFLP; ARMS	ITS	[137]
Alisma nanum	Rhizome	Dried	Yes	PCR, sequencing; RFLP; ARMS	ITS	[137]
Alisma orientale	Rhizome	Dried	Yes	PCR, sequencing; RFLP; ARMS	ITS	[137]
Alisma plantago-aquatica	Rhizome	Dried	Yes	PCR, sequencing; RFLP; ARMS	ITS	[137]
Alocasia macrorrhiza	Leaves	Fresh	Yes	PCR, sequencing; microarray (silicon)	5S gene spacer	[44]
Angelica acutiloba		Dried	Yes	PCR, sequencing	5S gene spacer	[107]
Angelica acutiloba var. acutiloba	Leaves	Fresh		PCR, sequencing	Spacer between atpF-atpA	[138]
Angelica acutiloba var. iwatensis	Leaves	Fresh		PCR, sequencing	Spacer between atpF-atpA	[138]
Angelica acutiloba var. sugijamae	Leaves	Fresh		PCR, sequencing	Spacer between atpF-atpA	[138]
Angelica acutiloba	Leaves	Fresh		RAPD; RFLP	N/A	[139]
Angelica acutiloba var. Sugiyamae	Leaves	Fresh		RAPD; RFLP	N/A	[139]
Angelica gigas		Dried	Yes	PCR, sequencing	5S gene spacer	[107]
Angelica sinensis		Dried	Yes	PCR, sequencing	5S gene spacer	[107]
Angelica sinensis	Root	Dried		RAPD; RFLP	N/A	[139]
Aralia elata			Yes	PCR, sequencing	ITS; trnC-trnD	[69]
Aralia franchetii			Yes	PCR, sequencing	ITS; trnC-trnD	[69]
Arisaema heterophyllum	Leaves	Fresh	Yes	PCR, sequencing; PCR-SR	Mannose-binding lectin	[121]
Artemisia aponica	Leaves	Fresh		PCR, sequencing, SCAR	N/A	[140]
Artemisia argyi	Leaves	Fresh		PCR, sequencing, SCAR	N/A	[140]
Artemisia capillaries	Leaves	Fresh		PCR, sequencing, SCAR	N/A	[140]
Artemisia iwayomogi	Leaves	Fresh		PCR, sequencing, SCAR	N/A	[140]
Artemisia keiskeana	Leaves	Fresh		PCR, sequencing, SCAR	N/A	[140]
Artemisia princes	Leaves	Fresh		PCR, sequencing, SCAR	N/A	[140]
Asarum arifolium			Yes	PCR, sequencing	ITS	[141]
Asarum asaroides			Yes	PCR, sequencing	ITS	[141]
Asarum asperum			Yes	PCR, sequencing	ITS	[141]
Asarum blumei			Yes	PCR, sequencing	ITS	[141]
Asarum canadense			Yes	PCR, sequencing	ITS	[141]
Asarum caudatum			Yes	PCR, sequencing	ITS	[141]
Asarum caudigerellum			Yes	PCR, sequencing	ITS	[141]
Asarum caudigerum			Yes	PCR, sequencing	ITS	[141]
Asarum caulescens			Yes	PCR, sequencing	ITS	[141]
Asarum crassum			Yes	PCR, sequencing	ITS	[141]
Asarum debile			Yes	PCR, sequencing	ITS	[141]
Asarum dimidiatum			Yes	PCR, sequencing	ITS	[21]
Asarum europaeum			Yes	PCR, sequencing	ITS	[141]

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Plant (scientific name)	Part	Condition	Voucher	Method	Gene	Ref
Asarum forbesii			Yes	PCR, sequencing	ITS	[141]
Asarum fudsinoi			Yes	PCR, sequencing	ITS	[141]
Asarum gelasinum			Yes	PCR, sequencing	ITS	[141]
Asarum hartwegii			Yes	PCR, sequencing	ITS	[141]
Asarum hatsushimae			Yes	PCR, sequencing	ITS	[141]
Asarum heterotropoides var. heterotropoides			Yes	PCR, sequencing	ITS	[21]
Asarum heterotropoides var. mandshuricum				PCR, sequencing	ITS	[142]
Asarum heterotropoides var. mandshuricum			Yes	PCR, sequencing	ITS	[21]
Asarum heterotro-poides var. seoulense			Yes	PCR, sequencing	ITS	[21]
Asarum himalaicum			Yes	PCR, sequencing	ITS	[141]
Asarum lemonii			Yes	PCR, sequencing	ITS	[141]
Asarum marmoratum			Yes	PCR, sequencing	ITS	[141]
Asarum maruyamae			Yes	PCR, sequencing	ITS	[21]
Asarum mikuniense			Yes	PCR, sequencing	ITS	[21]
Asarum minimitanianum			Yes	PCR, sequencing	ITS	[141]
Asarum minor			Yes	PCR, sequencing	ITS	[141]
Asarum misandrum			Yes	PCR, sequencing	ITS	[21]
Asarum patens			Yes	PCR, sequencing	ITS	[21]
Asarum pulchellum			Yes	PCR, sequencing	ITS	[141]
Asarum satsumense			Yes	PCR, sequencing	ITS	[141]
Asarum savatieri			Yes	PCR, sequencing	ITS	[141]
Asarum shuttleworthii			Yes	PCR, sequencing	ITS	[141]
Asarum sieboldii				PCR, sequencing	ITS	[142]
Asarum sieboldii			Yes	PCR, sequencing	ITS	[141]
Asarum sieboldii f. maculatum			Yes	PCR, sequencing	ITS	[21]
Asarum sieboldii f. seoulense				PCR, sequencing	ITS	[142]
Asarum sieboldii f. siboldii			Yes	PCR, sequencing	ITS	[21]
Asarum sieboldii var. cornutum			Yes	PCR, sequencing	ITS	[21]
Asarum speciosum			Yes	PCR, sequencing	ITS	[141]
Asarum takaoi			Yes	PCR, sequencing	ITS	[141]
Asarum tohokuense			Yes	PCR, sequencing	ITS	[21]
Asarum versicolor			Yes	PCR, sequencing	ITS	[21]
Asarum virginicum			Yes	PCR, sequencing	ITS	[141]
Asarum yakusimense			Yes	PCR, sequencing	ITS	[141]
Astragalus aksuensis		Dried	Yes	PCR, sequencing	5S gene spacer, ITS; 18S rRNA	[20]
Astragalus austrosibiricus		Dried	Yes	PCR, sequencing	5S gene spacer; ITS; 18S rRNA	[20]
Astragalus hoantchy		Dried	Yes	PCR, sequencing	5S gene spacer; ITS; 18S rRNA	[20]
Astragalus hoantchy subsp. Dshimensis		Dried	Yes	PCR, sequencing	5S gene spacer; ITA; 18S rRNA	[20]
Astragalus lehmannianus	Leaves, roots	Fresh, crude drug	Yes	PCR, sequencing	5S gene spacer	[104]
Astragalus lehmannianus		Dried	Yes	PCR, sequencing	5S gene spacer; ITS; 18S rRNA	[20]
Astragalus lepsensis		Dried	Yes	PCR, sequencing	5S gene spacer; ITS; 18S rRNA	[20]
Astragalus membranaceus	Leaves, roots	Fresh, crude drug	Yes	PCR, sequencing	5S gene spacer	[104]
		Dried	Yes	PCR, sequencing	5S gene spacer; ITS; 18S rRNA	[20]

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Plant (scientific name)	Part	Condition	Voucher	Method	Gene	Ref
	Roots	Fresh		3′untranslated region sequence-based amplified polymorphism (UAP)	3′untranslated regions (3′UTR)	[102]
				RAPD	N/A	[106]
Astragalus membranaceus from 23 locations		Dried		AP-PCR	ITS	[105]
Astragalus membranaceus var. mongholicus	Leaves, roots	Fresh, crude drug	Yes	PCR, sequencing	5S gene spacer	[104]
		Dried	Yes	PCR, sequencing	5S gene spacer; ITS; 18S rRNA	[20]
	Roots	Fresh		3′untranslated region sequence-based amplified polymorphism (UAP)	3′untranslated regions (3′UTR)	[102]
Astragalus membranaceus var. mongholicus from 23 locations		Dried		AP-PCR	ITS	[105]
Astragalus propinquus		Dried	Yes	PCR, sequencing	5S gene spacer; ITS; 18S rRNA	[20]
Astragalus sieversianus		Dried	Yes	PCR, sequencing	5S gene spacer; ITS; 18S rRNA	[20]
Astraglus hoantchy	Leaves, roots	Fresh, crude drug	Yes	PCR, sequencing	5S gene spacer	[104]
Atractylodes chinensis	Crude drug			PCR, sequencing	ITS	[143]
Atractylodes japonica				RAPD	N/A	[144]
Atractylodes japonica	Crude drug			PCR, sequencing	ITS	[143]
Atractylodes lancea				RAPD	N/A	[144]
Atractylodes lancea	Leaves	Fresh		PCR, sequencing, SCAR	N/A	[140]
Atractylodes ovata				RAPD	N/A	[144]
Atractylodes ovata	Crude drug			PCR, sequencing	ITS	[143]
Bacopa monnieri	Leaves	Fresh		RAPD	N/A	[145]
Bupleurum aureum		Fresh		PCR, sequencing	ITS	[146]
Bupleurum chinense		Fresh		PCR, sequencing	ITS	[146]
Bupleurum commelynoideium var. flaviflorum		Fresh		PCR, sequencing	ITS	[146]
Bupleurum krylovianum		Fresh		PCR, sequencing	ITS	[146]
Bupleurum longiradiatum		Fresh		PCR, sequencing	ITS	[146]
Bupleurum marginatum var. stenophyllum		Fresh		PCR, sequencing	ITS	[146]
Bupleurum scorzonerifolium		Fresh		PCR, sequencing	ITS	[146]
Bupleurum sibiricum		Fresh		PCR, sequencing	ITS	[146]
Bupleurum smithii		Fresh		PCR, sequencing	ITS	[146]
Bupleurum tianschanicum		Fresh		PCR, sequencing	ITS	[146]
Bupleurum yinchouwense		Fresh		PCR, sequencing	ITS	[146]
Cannabis sativa	Leaves	Fresh	Yes	ISSR	N/A	[88]
	Leaves, stems, flowering heads	Fresh, dried		RAPD	N/A	[87]
	Leaves, inflor-escences	Fresh, dried		AFLP	N/A	[86]
Carthamus tinctorius	Leaf	Fresh, crude drug	Yes	PCR, sequencing	5S gene spacer	[147]
Changium smyrnioides	Leaves	Dried	Yes	RAPD	N/A	[148]
Codonopsis pilulosa	Roots	Dried	Yes	AP-PCR, RAPD	N/A	[109]
Corton tiglium	Leaves	Fresh	Yes	PCR, sequencing; microarray (silicon)	5S gene spacer	[44]
Crocus sativus	Leaf	Fresh, crude drug	Yes	PCR, sequencing	5S gene spacer	[147]

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Plant (scientific name)	Part	Condition	Voucher	Method	Gene	Ref
Cultivated Ephedra	Stem	Fresh	Yes	PCR, sequencing	ITS; trnL; trnL-trnF	[83]
Curcuma chuanyujin		Dried, crude drug	Yes	PCR, sequencing	5S gene spacer	[108]
Curcuma kwangsiensis		Dried, crude drug	Yes	PCR, sequencing	5S gene spacer	[108]
Curcuma longa		Dried, crude drug	Yes	PCR, sequencing	5S gene spacer	[108]
Curcuma phaeocaulis		Dried, crude drug	Yes	PCR, sequencing	5S gene spacer	[108]
Curcuma wenyujin		Dried, crude drug	Yes	PCR, sequencing	5S gene spacer	[108]
Datura inoxia	Leaves	Fresh	Yes	PCR, sequencing; microarray (silicon)	5S gene spacer	[44]
Datura metel	Leaves	Fresh	Yes	PCR, sequencing; microarray (silicon)	5S gene spacer	[44]
Datura tatula	Leaves	Fresh	Yes	PCR, sequencing; microarray (silicon)	5S gene spacer	[44]
Dendrobium acinaforme	Leaves, stems	Fresh, dried		PCR	ITS	[91]
Dendrobium aduncum	Stem	Fresh	Yes	PCR, sequencing	ITS	[94]
	Leaves, stems	Fresh, dried		PCR	ITS	[91]
Dendrobium aphyllum	Leaves, stems	Fresh, dried		PCR	ITS	[91]
				PCR, sequencing	ITS	[93]
Dendrobium aurantiacum	Leaves, stems	Fresh, dried		PCR	ITS	[91]
Dendrobium aurantiacum var. denneanum	Leaves, stems	Fresh, dried		PCR	ITS	[91]
Dendrobium auriantiacum	Stems			Microarray (nylon)	gDNA	[95]
Dendrobium brymerianum	Leaves, stems	Fresh, dried		PCR	ITS	[91]
Dendrobium candidum (= Dendrobium officinale)	Stem	Fresh	Yes	PCR, sequencing	ITS	[94]
		Fresh, medicinal formulation	Yes	PCR, microarray (glass)	ITS	[101]
Dendrobium cantonensis	Stem	Fresh	Yes	PCR, sequencing	ITS	[94]
Dendrobium capillipes	Leaves, stems	Fresh, dried		PCR	ITS	[91]
Dendrobium cariniferum	Leaves, stems	Fresh, dried		PCR	ITS	[91]
Dendrobium chrysanthum	Stem	Fresh	Yes	PCR, sequencing	ITS	[94]
	Leaves, stems	Fresh, dried		PCR	ITS	[91]
		Fresh, medicinal formulation	Yes	PCR, microarray (glass)	ITS	[101]
				PCR, sequencing	ITS	[93]
Dendrobium chrysotoxum	Stem	Fresh	Yes	PCR, sequencing	ITS	[94]
	Leaves, stems	Fresh, dried		PCR	ITS	[91]
		Fresh, medicinal formulation	Yes	PCR, microarray (glass)	ITS	[101]
	Stems			Microarray (nylon)	gDNA	[95]
Dendrobium crepidatum	Stem	Fresh	Yes	PCR, sequencing	ITS	[94]
	Leaves, stems	Fresh, dried		PCR	ITS	[91]
				PCR, sequencing	ITS	[93]
Dendrobium crystallinum	Leaves, stems	Fresh, dried		PCR	ITS	[91]
		Fresh, medicinal formulation	Yes	PCR, microarray (glass)	ITS	[101]
Dendrobium densiflorum	Stem	Fresh	Yes	PCR, sequencing	ITS	[94]
	Leaves, stems	Fresh, dried		PCR	ITS	[91]
Dendrobium densiflorum		Fresh, medicinal formulation	Yes	PCR, microarray (glass)	ITS	[101]

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Plant (scientific name)	Part	Condition	Voucher	Method	Gene	Ref
	Leaves, stems	Fresh, dried	Yes		ITS	[91]
	Leaves, stems	Fresh, dried		PCR	ITS	[91]
Dendrobium ellipsophyllum	Leaves, stems	Fresh, dried		PCR	ITS	[91]
Dendrobium exile	Leaves, stems	Fresh, dried		PCR	ITS	[91]
Dendrobium falconeri	Leaves, stems	Fresh, dried		PCR	ITS	[91]
		Fresh, medicinal formulation	Yes	PCR, microarray (glass)	ITS	[101]
Dendrobium fimbriatum	Stem	Fresh	Yes	PCR, sequencing	ITS	[94]
	Leaves, stems	Fresh, dried		PCR	ITS	[91]
		Fresh, medicinal formulation	Yes	PCR, microarray (glass)	ITS	[101]
	Stems			Microarray (nylon)	gDNA	[95]
Dendrobium fimbriatum var. occulatum	Stem	Fresh	Yes	PCR, sequencing	ITS	[94]
Dendrobium findlayanum	Leaves, stems	Fresh, dried		PCR	ITS	[91]
Dendrobium flexicaule	Leaves, stems	Fresh, dried		PCR	ITS	[91]
Dendrobium funiushanense	Leaves, stems	Fresh, dried		PCR	ITS	[91]
Dendrobium gratiosissimum	Leaves, stems	Fresh, dried	Yes		ITS	[91]
	Leaves, stems	Fresh, dried		PCR	ITS	[91]
Dendrobium hancockii	Leaves, stems	Fresh, dried		PCR	ITS	[91]
Dendrobium henanense	Leaves, stems	Fresh, dried		PCR	ITS	[91]
Dendrobium hercoglossum	Stem	Fresh	Yes	PCR, sequencing	ITS	[94]
	Leaves, stems	Fresh, dried		PCR	ITS	[91]
Dendrobium huoshanense	Leaves, stems	Fresh, dried		PCR	ITS	[91]
Dendrobium jenkinsii	Stem	Fresh	Yes	PCR, sequencing	ITS	[94]
		Fresh, medicinal formulation	Yes	PCR, microarray (glass)	ITS	[101]
Dendrobium lindleyi	Stem	Fresh	Yes	PCR, sequencing	ITS	[94]
		Fresh, medicinal formulation	Yes	PCR, microarray (glass)	ITS	[101]
Dendrobium lituiflorum	Leaves, stems	Fresh, dried		PCR	ITS	[91]
				PCR, sequencing	ITS	[93]
Dendrobium loddigesii	Stem	Fresh	Yes	PCR, sequencing	ITS	[94]
	Leaves, stems	Fresh, dried	Yes		ITS	[91]
	Leaves, stems	Fresh, dried		PCR	ITS	[91]
		Fresh, medicinal formulation	Yes	PCR, microarray (glass)	ITS	[101]
Dendrobium lohohense	Stem	Fresh	Yes	PCR, sequencing	ITS	[94]
	Leaves, stems	Fresh, dried		PCR	ITS	[91]
		Fresh, medicinal formulation	Yes	PCR, microarray (glass)	ITS	[101]
Dendrobium miniliforme	Leaves, stems	Fresh, dried		PCR	ITS	[91]
Dendrobium moniliforme	Stem	Fresh	Yes	PCR, sequencing	ITS	[94]
		Fresh, medicinal formulation	Yes	PCR, microarray (glass)	ITS	[101]
Dendrobium moschatum	Stem	Fresh	Yes	PCR, sequencing	ITS	[94]
		Fresh, medicinal formulation	Yes	PCR, microarray (glass)	ITS	[101]

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Plant (scientific name)	Part	Condition	Voucher	Method	Gene	Ref
Dendrobium nobile	Stem	Fresh	Yes	PCR, sequencing	ITS	[94]
	Leaves, stems	Fresh, dried		PCR	ITS	[91]
		Fresh, medicinal formulation	Yes	PCR, microarray (glass)	ITS	[101]
	Stems			Microarray (nylon)	gDNA	[95]
Dendrobium officinale	Leaves, stems	Fresh, dried	Yes		ITS	[91]
	Leaves, stems	Fresh, dried		PCR	ITS	[91]
	Stems			Microarray (nylon)	gDNA	[95]
	Stems, leaves	Fresh, Dried		PCR, sequencing	ITS	[90]
Dendrobium pendulum	Leaves, stems	Fresh, dried		PCR	ITS	[91]
		Fresh, medicinal formulation	Yes	PCR, microarray (glass)	ITS	[101]
Dendrobium primulinum	Leaves, stems	Fresh, dried		PCR	ITS	[91]
		Fresh, medicinal formulation	Yes	PCR, microarray (glass)	ITS	[101]
				PCR, sequencing	ITS	[93]
Dendrobium salaccense	Leaves, stems	Fresh, dried		PCR	ITS	[91]
Dendrobium thyrsiflorum	Leaves, stems	Fresh, dried		PCR	ITS	[91]
Dendrobium wardianum	Leaves, stems	Fresh, dried		PCR	ITS	[91]
Dendrobium williamsonii	Stem	Fresh	Yes	PCR, sequencing	ITS	[94]
	Leaves, stems	Fresh, dried		PCR	ITS	[91]
Digitalis obscura	Leaves	Fresh		RAPD		[149]
Dioscorea alata				PCR, sequencing	18S rRNA	[150]
Dioscorea japonica				PCR, sequencing	18S rRNA	[150]
Dioscorea persimilis				PCR, sequencing	18S rRNA	[150]
Dioscorea polystachia				PCR, sequencing	18S rRNA	[150]
Dysosma aurantiocaulis	Leaves	Dried	Yes	PCR, RFLP	trnT-trnL; trnD-trnT	[151]
Dysosma difformis	Leaves	Dried	Yes	PCR, RFLP	trnT-trnL; trnD-trnT	[151]
Dysosma majorensis	Leaves	Dried	Yes	PCR, RFLP	trnT-trnL; trnD-trnT	[151]
Dysosma pleiantha				PCR, sequencing; microarray (silicon)	5S gene spacer	[44]
	Leaves	Dried	Yes	PCR, RFLP	trnT-trnL; trnD-trnT	[151]
Dysosma veitchii	Leaves	Dried	Yes	PCR, RFLP	trnT-trnL; trnD-trnT	[151]
Dysosma versipellis		Fresh		PCR, sequencing; microarray (silicon)	5S gene spacer	[44]
	Leaves	Dried	Yes	PCR, RFLP	trnT-trnL; trnD-trnT	[151]
Echinacea angustifolia	Leaves			RAPD	N/A	[152]
				RAPD	N/A	[153]
Echinacea artrorubens				RAPD	N/A	[152]
Echinacea pallida				RAPD	N/A	[152]
				RAPD	N/A	[153]
Echinacea purpurea				RAPD	N/A	[152]
				RAPD	N/A	[153]
Ephedra antisyphilitca	Aerial parts	Dried	Yes	PCR, sequencing	pbsA-trnH	[85]
Ephedra aspera	Aerial parts	Dried	Yes	PCR, sequencing	psbA-trnH	[85]
Ephedra californica	Aerial parts	Dried	Yes	PCR, sequencing	psbA-trnH	[85]
Ephedra coryi	Aerial parts	Dried	Yes	PCR, sequencing	psbA-trnH	[85]
Ephedra distachya	Aerial parts	Dried	Yes	PCR, sequencing	psbA-trnH	[85]
Ephedra equisetina		Dried, crude drug	Yes	PCR, sequencing; PCR, RFLP	chlB; ITS	[81]
	Stem	Fresh	Yes	PCR, sequencing	ITS; trnL; trnL-trnF	[84]
	Aerial parts	Dried	Yes	PCR, sequencing	psbA-trnH	[85]
Ephedra fasciculata	Aerial parts	Dried	Yes	PCR, sequencing	psbA-trnH	[85]
Ephedra fragilis	Aerial parts	Dried	Yes	PCR, sequencing	psbA-trnH	[85]
Ephedra fedtschenkkoae	Aerial parts	Dried	Yes	PCR, sequencing	psbA-trnH	[85]
Ephedra foeminea	Aerial parts	Dried	Yes	PCR, sequencing	psbA-trnH	[85]

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Plant (scientific name)	Part	Condition	Voucher	Method	Gene	Ref
Ephedra frustilata	Aerial parts	Dried	Yes	PCR, sequencing	psbA-trnH	[85]
Ephedra gerardiana	Stem	Fresh	Yes	PCR, sequencing	ITS; trnL; trnL-trnF	[84]
Ephedra intermedia		Dried, crude drug	Yes	PCR, sequencing; PCR, RFLP	chlB; ITS	[81]
	Stem	Fresh	Yes	PCR, sequencing	ITS; trnL; trnL-trnF	[84]
Ephedra likiangensis	Stem	Fresh	Yes	PCR, sequencing	ITS; trnL; trnL-trnF	[84]
	Aerial parts	Dried	Yes	PCR, sequencing	psbA-trnH	[85]
Ephedra major	Aerial parts	Dried	Yes	PCR, sequencing	psbA-trnH	[85]
Ephedra minuta	Stem	Fresh	Yes	PCR, sequencing	ITS; trnL; trnL-trnF	[84]
Ephedra monosperma	Stem	Fresh	Yes	PCR, sequencing	ITS; trnL; trnL-trnF	[84]
Ephedra nevadensis	Aerial parts	Dried	Yes	PCR, sequencing	psbA-trnH	[85]
Ephedra ochreata	Aerial parts	Dried	Yes	PCR, sequencing	psbA-trnH	[85]
Ephedra przewalskii		Dried, crude drug	Yes	PCR, sequencing; RFLP	chlB; ITS	[81]
	Stem	Fresh	Yes	PCR, sequencing	ITS; trnL; trnL-trnF	[84]
	Aerial parts	Dried	Yes	PCR, sequencing	psbA-trnH	[85]
Ephedra saxatilis	Aerial parts	Dried	Yes	PCR, sequencing	psbA-trnH	[85]
Ephedra sinica		Dried, crude drug	Yes	PCR, sequencing; PCR, RFLP	chlB; ITS	[81]
	Stem	Fresh	Yes	PCR, sequencing	ITS; trnL; trnL-trnF	[84]
	Aerial parts	Dried	Yes	PCR, sequencing	psbA-trnH	[85]
Ephedra trifurca	Aerial parts	Dried	Yes	PCR, sequencing	psbA-trnH	[85]
Ephedra torreyana	Aerial parts	Dried	Yes	PCR, sequencing	psbA-trnH	[85]
Ephedra viridis	Aerial parts	Dried	Yes	PCR, sequencing	psbA-trnH	[85]
Epimedioum brevicornu			Yes	PCR, sequencing	5S gene spacer	[154]
Epimedium koreanum			Yes	PCR, sequencing	5S gene spacer	[154]
Epimedium pubescens			Yes	PCR, sequencing	5S gene spacer	[154]
Epimedium sagittatum			Yes	PCR, sequencing	5S gene spacer	[154]
Epimedium wushanense			Yes	PCR, sequencing	5S gene spacer	[154]
Euphorbia discolor			Yes	PCR, sequencing	ITS	[155]
Euphorbia esula			Yes	PCR, sequencing	ITS	[155]
Euphorbia kansui	Leaves	Fresh	Yes	PCR, sequencing; microarray (silicon)	5S gene spacer	[44]
Euphorbia lamprocarpa			Yes	PCR, sequencing	ITS	[155]
Euphorbia lathyris			Yes	PCR, sequencing	ITS	[155]
Euphorbia pekinensis			Yes	PCR, sequencing	ITS	[155]
Euphorbia peplus			Yes	PCR, sequencing	ITS	[155]
Euphorbia turczaninowii			Yes	PCR, sequencing	ITS	[155]
Fritillaria anhuiensis	Leaves, bulbs	Fresh	Yes	PCR, sequencing; restriction digest	5S gene spacer	[77]
Fritillaria cirrhosa	Leaves, bulbs	Fresh	Yes	PCR, sequencing; restriction digest	5S gene spacer	[77]
	Leaves, bulbs	Dried	Yes	PCR, sequencing; PCR, RFLP	ITS	[80]
				PCR, sequencing; PCR, microarray (glass)	26S rRNA	[79]
Fritillaria delavayi	Leaves, bulbs	Dried	Yes	PCR, sequencing; PCR, RFLP	ITS	[80]
				PCR, sequencing; PCR, microarray (glass)	26S rRNA	[64]
Fritillaria hupehensis	Leaves, bulbs	Dried	Yes	PCR, sequencing; PCR, RFLP	ITS	[80]
Fritillaria pallidiflora	Leaves, bulbs	Dried	Yes	PCR, sequencing; PCR, RFLP	ITS	[80]
				PCR, sequencing; PCR, microarray (glass)	26S rRNA	[64]
Fritillaria przewalskii	Leaves, bulbs	Dried	Yes	PCR, sequencing; PCR, RFLP	ITS	[80]
Fritillaria puqiensis	Leaves, bulbs	Fresh	Yes	PCR, sequencing; restriction digest	5S gene spacer	[77]
	Leaves, bulbs	Dried	Yes	PCR, sequencing; PCR, RFLP	ITS	[80]
Fritillaria thunbergii	Leaves, bulbs	Fresh	Yes	PCR, sequencing; restriction digest	5S gene spacer	[77]
	Leaves, bulbs	Dried	Yes	PCR, sequencing; PCR, RFLP	ITS	[80]
Fritillaria thunbergii var. chekiangensis				PCR, sequencing; PCR, microarray (glass)	26S rRNA	[64]
Fritillaria unibracteata	Leaves, bulbs	Dried	Yes	PCR, sequencing; PCR, RFLP	ITS	[80]

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Plant (scientific name)	Part	Condition	Voucher	Method	Gene	Ref
				PCR, sequencing; PCR, microarray (glass)	26S rRNA	[64]
Fritillaria ussurensis	Leaves, bulbs	Dried	Yes	PCR, sequencing; PCR, RFLP	ITS	[80]
				PCR, sequencing; PCR, microarray (glass)	26S rRNA	[64]
Fritillaria walujewii	Leaves, bulbs	Dried	Yes	PCR, sequencing; PCR, RFLP	ITS	[80]
Gentiana straminea			Yes	PCR, sequencing; allele-specific diagnostic PCR	rpl16; ITS	[156]
Glehnia littoralis	Leaves	Fresh		RFLP	N/A	[157]
		Fresh, crude drug	Yes	PCR, sequencing	5S gene spacer	[135]
Gnetum gnemon	Aerial parts	Fresh	Yes	PCR, sequencing	psbA-trnH	[85]
Gnetum lepostachyum	Stems	Fresh	Yes	PCR, sequencing	ITS; trnL; trnL-trnF	[84]
Halenia elliptica			Yes	PCR, sequencing; allele-specific diagnostic PCR	rpl16; ITS	[156]
Hedysarum polybotris	Leaves, roots	Fresh, crude drug	Yes	PCR, sequencing	5S gene spacer	[104]
				RAPD	N/A	[106]
Hemerocallis citrina	Leaf	Fresh, crude drug	Yes	PCR, sequencing	5S gene spacer	[147]
Hermerocallis fulva	Leaf	Fresh, crude drug	Yes	PCR, sequencing	5S gene spacer	[147]
Humulus hops	Leaves, stems, flowering heads	Fresh, dried		RAPD	N/A	[87]
Hyoscyamus niger	Leaves	Fresh	Yes	PCR, sequencing; microarray (silicon)	5S gene spacer	[44]
Lamium amplexicaule				PCR, sequencing	ITS	[158]
Leonurus chaituroides				PCR, sequencing	ITS	[158]
Leonurus heterophyllus				PCR, sequencing	ITS	[158]
Leonurus pseudomacranthus				PCR, sequencing	ITS	[158]
Leonurus sibiricus				PCR, sequencing	ITS	[158]
Ligularia dentata			Yes	PCR, sequencing	5S gene spacer	[159]
Ligularia knaitzensis			Yes	PCR, sequencing	5S gene spacer	[159]
Ligularia lankongensis			Yes	PCR, sequencing	5S gene spacer	[159]
Ligularia lapathifolia			Yes	PCR, sequencing	5S gene spacer	[159]
Ligularia narynensis			Yes	PCR, sequencing	5S gene spacer	[159]
Ligularia nelumbifolia			Yes	PCR, sequencing	5S gene spacer	[159]
Ligularia pleurocaulis			Yes	PCR, sequencing	5S gene spacer	[159]
Ligularia przewalskii			Yes	PCR, sequencing	5S gene spacer	[159]
Ligularia sagitta			Yes	PCR, sequencing	5S gene spacer	[159]
Ligularia subspicata			Yes	PCR, sequencing	5S gene spacer	[159]
Ligularia tongolensis			Yes	PCR, sequencing	5S gene spacer	[159]
Ligularia virgaurea			Yes	PCR, sequencing	5S gene spacer	[159]
Lomatogonium oreacharis			Yes	PCR, sequencing; allele-specific diagnostic PCR	rpl16; ITS	[156]
Lycium barbarum	Fruit	Dried	Yes	RAPD	N/A	[160]
Lycium barbarum cv. "Tianjinense"	Fruit	Dried	Yes	RAPD	N/A	[160]
Lycium barbarum var. aranticarpum	Fruit	Dried	Yes	RAPD	N/A	[160]
Lycium barbarum var. potaninii	Fruit	Dried	Yes	RAPD	N/A	[160]
Lycium chinense	Fruit	Dried	Yes	RAPD	N/A	[160]
Lycium dasy Stemsum var. rubricaulium	Fruit	Dried	Yes	RAPD	N/A	[160]
Lycium ruthenicum	Fruit	Dried	Yes	RAPD	N/A	[160]
Lycium truncatum	Fruit	Dried	Yes	RAPD	N/A	[160]
Medicago sativa	Leaves; dried ground material	Fresh, dried		PCR, sequencing; RFLP	ITS	[161]
Mirablis jalapa	Roots	Fresh, dried	Yes	AP-PCR; RAPD	N/A	[71]
Nandina domestica			Yes	PCR, sequencing	5S gene spacer	[154]
Panax assamicus			Yes	PCR, sequencing	ITS; trnC-trnD	[69]
Panax bipinnatifidus var. angustifolius			Yes	PCR, sequencing	ITS; trnC-trnD	[69]

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Plant (scientific name)	Part	Condition	Voucher	Method	Gene	Ref
Panax bipinnatifidus var. bipinnatifidus			Yes	PCR, sequencing	ITS; trnC-trnD	[69]
Panax elegantior			Yes	PCR, sequencing	ITS; trnC-trnD	[69]
Panax ginseng	Roots			AP-PCR	N/A	[17]
	Roots	Fresh, dried	Yes	AP-PCR; RAPD	N/A	[71]
	Roots	Fresh, dried	Yes	RAPD, sequencing; SCAR	N/A	[162]
	Roots	Fresh, dried	Yes	RAPD, DALP, sequencing	N/A	[66]
	Leaves, roots	Fresh, crude drug	Yes	MARMS	trnK, 18S rRNA	[74]
				PCR	SSR	[163]
		Crude drug		RAPD	N/A	[72]
			Yes	PCR, sequencing	ITS; trnC-trnD	[69]
Panax japonicus	Leaves, roots	Fresh, crude drug	Yes	MARMS	trnK, 18S rRNA	[74]
		Crude drug		RAPD	N/A	[72]
			Yes	PCR, sequencing	ITS; trnC-trnD	[69]
Panax major			Yes	PCR, sequencing	ITS; trnC-trnD	[69]
Panax notoginseng	Roots	Fresh, dried	Yes	AP-PCR; RAPD	N/A	[71]
	Leaves, roots	Fresh, crude drug	Yes	MARMS	trnK, 18S rRNA	[74]
		Crude drug		RAPD	N/A	[72]
			Yes	PCR, sequencing	ITS; trnC-trnD	[69]
	Roots	Fresh		AFLP; PCR, sequencing	ITS 2	[67]
Panax omeiensis			Yes	PCR, sequencing	ITS; trnC-trnD	[69]
Panax pseudoginseng			Yes	PCR, sequencing	ITS; trnC-trnD	[69]
Panax quinquefolium	Roots			AP-PCR	N/A	[17]
	Roots	Fresh, dried	Yes	AP-PCR; RAPD	N/A	[71]
	Roots	Fresh, dried	Yes	RAPD, sequencing; SCAR	N/A	[162]
	Roots	Fresh, dried	Yes	RAPD, DALP, sequencing	N/A	[66]
	Leaves, roots	Fresh, crude drug	Yes	MARMS	trnK, 18S rRNA	[74]
				PCR	Microsatellite marker	[163]
Panax quinquefolius		Crude drug		RAPD	N/A	[72]
			Yes	PCR, sequencing	ITS; trnC-trnD	[69]
Panax shangianus			Yes	PCR, sequencing	ITS; trnC-trnD	[69]
Panax sinensis			Yes	PCR, sequencing	ITS; trnC-trnD	[69]
Panax stipulenatus			Yes	PCR, sequencing	ITS; trnC-trnD	[69]
Panax trifolius			Yes	PCR, sequencing	ITS; trnC-trnD	[69]
Panax variabilis			Yes	PCR, sequencing	ITS; trnC-trnD	[69]
Panax vietnamensis	Leaves, roots	Fresh, crude drug	Yes	MARMS	trnK, 18S rRNA	[74]
			Yes	PCR, sequencing	ITS; trnC-trnD	[69]
Panax wangianus			Yes	PCR, sequencing	ITS; trnC-trnD	[69]
Panax zingiberensis			Yes	PCR, sequencing	ITS; trnC-trnD	[69]
Perilla frutescens				PCR, sequencing	ITS	[164]
Perilla frutescens var. arguta				PCR, sequencing	ITS	[164]
Perilla frutescens var. auriculato-dentata				PCR, sequencing	ITS	[164]
Perilla frutescens var. crispa				PCR, sequencing	ITS	[164]
Pholidota cantonensis	Stems	Fresh	Yes	PCR, sequencing	ITS	[94]
Phyllanthus amarus			Yes	PCR, sequencing; multiplex PCR	ITS; atpB; rbcL	[165]
Phyllanthus arenarius			Yes	PCR, sequencing; multiplex PCR	ITS; atpB; rbcL	[165]
Phyllanthus calcynus			Yes	PCR, sequencing; multiplex PCR	ITS; atpB; rbcL	[165]
Phyllanthus clakei			Yes	PCR, sequencing; multiplex PCR	ITS; atpB; rbcL	[165]
Phyllanthus cochinchinensis			Yes	PCR, sequencing; multiplex PCR	ITS; atpB; rbcL	[165]
Phyllanthus distichus	Leaves	Fresh		RAPD, sequencing SCAR	N/A	[166]
Phyllanthus emblica (= Emblica officinalis)	Leaves	Fresh and dried		RAPD, sequencing; SCAR	N/A	[166]
Phyllanthus emblica (= Emblica officinalis)			Yes	PCR, sequencing; multiplex PCR	ITS; atpB; rbcL	[165]
Phyllanthus flexuosus			Yes	PCR, sequencing; multiplex PCR	ITS; atpB; rbcL	[165]

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Plant (scientific name)	Part	Condition	Voucher	Method	Gene	Ref
Phyllanthus glaucus			Yes	PCR, sequencing; multiplex PCR	ITS; atpB; rbcL	[165]
Phyllanthus guangdongensis			Yes	PCR, sequencing; multiplex PCR	ITS; atpB; rbcL	[165]
Phyllanthus hainanensis			Yes	PCR, sequencing; multiplex PCR	ITS; atpB; rbcL	[165]
Phyllanthus indofischeri	Leaves	Fresh		RAPD, sequencing; SCAR	N/A	[166]
Phyllanthus lokohensis			Yes	PCR, sequencing; multiplex PCR	ITS; atpB; rbcL	[165]
Phyllanthus myrtifolius			Yes	PCR, sequencing; multiplex PCR	ITS; atpB; rbcL	[165]
Phyllanthus niruri	Leaves	Fresh		RAPD, sequencing; SCAR,	N/A	[166]
			Yes	PCR, sequencing; multiplex PCR	ITS; atpB; rbcL	[165]
Phyllanthus nummulariifolius			Yes	PCR, sequencing; multiplex PCR	ITS; atpB; rbcL	[165]
Phyllanthus parvifolius			Yes	PCR, sequencing; multiplex PCR	ITS; atpB; rbcL	[165]
Phyllanthus reticulatus	Leaves	Fresh		RAPD, sequencing; SCAR	N/A	[166]
Phyllanthus reticulatus			Yes	PCR, sequencing; multiplex PCR	ITS; atpB; rbcL	[165]
Phyllanthus ruber			Yes	PCR, sequencing; multiplex PCR	ITS; atpB; rbcL	[165]
Phyllanthus simplex	Leaves	Fresh		RAPD, sequencing; SCAR	N/A	[166]
Phyllanthus taxodiifolius			Yes	PCR, sequencing; multiplex PCR	ITS; atpB; rbcL	[165]
Phyllanthus urinaria	Leaves	Fresh		RAPD, sequencing; SCAR	N/A	[166]
			Yes	PCR, sequencing; multiplex PCR	ITS; atpB; rbcL	[165]
Phyllanthus ussuriensis			Yes	PCR, sequencing; multiplex PCR	ITS; atpB; rbcL	[165]
Phyllanthus virgatus			Yes	PCR, sequencing; multiplex PCR	ITS; atpB; rbcL	[165]
Phytolacca acinosa	Roots	Fresh, dried	Yes	AP-PCR; RAPD	N/A	[71]
Pinellia cordata	Leaves	Fresh	Yes	PCR, sequencing; microarray (silicon)	5S gene spacer	[44]
Pinellia pedatisecta	Leaves	Fresh	Yes	PCR, sequencing; microarray (silicon)	5S gene spacer	[44]
	Leaves	Fresh	Yes	PCR, sequencing; PCR-SR	Mannose-binding lectin	[121]
Pinellia pedatisecta				PCR, sequencing	18S rRNA	[167]
				PCR, sequencing	18S rRNA	[153]
Pinellia ternata	Leaves	Fresh	Yes	PCR, sequencing; microarray (silicon)	5S gene spacer	[44]
	Leaves	Fresh	Yes	PCR, sequencing; PCR-SR	Mannose-binding lectin	[121]
				RAPD	N/A	[168]
				PCR, sequencing	18S rRNA	[167]
				PCR, sequencing	18S rRNA	[153]
Plantago ovata	Seedlings	Fresh		RAPD	N/A	[169]
Platicodon grandiflorum	Roots	Fresh, dried	Yes	AP-PCR; RAPD	N/A	[71]
Plectranthus barbatus	Leaves		Yes	AFLP	N/A	[170]
Plectranthus grandis	Leaves		Yes	AFLP	N/A	[170]
Plectranthus ornatus	Leaves		Yes	AFLP	N/A	[170]
Pogostemon cablin			Yes	PCR, sequencing	18S rRNA; matK	[136]
Pueraria lobata			Yes	PCR, sequencing	ITS; 5S gene spacer	[171]
Pueraria montana			Yes	PCR, sequencing	ITS; 5S gene spacer	[171]
Pueraria thomsonii			Yes	PCR, sequencing	ITS; 5S gene spacer	[171]
Pulsatilla vulgaris	Leaves		Yes	AFLP	N/A	[134]
Rehmannia chingii	Leaves	Dried	Yes	PCR, sequencing	ITS, trnL-trnF, rps16	[18]
Rehmannia elata	Leaves	Dried	Yes	PCR, sequencing	ITS, trnL-trnF, rps16	[18]
Rehmannia glutinosa	Leaves	Dried	Yes	PCR, sequencing	ITS, trnL-trnF, rps16	[18]

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Plant (scientific name)	Part	Condition	Voucher	Method	Gene	Ref
Rehmannia henryi	Leaves	Dried	Yes	PCR, sequencing	ITS, trnL-trnF, rps16	[18]
Rehmannia piasezkii	Leaves	Dried	Yes	PCR, sequencing	ITS, trnL-trnF, rps16	[18]
Rehmannia solanifolia	Leaves	Dried	Yes	PCR, sequencing	ITS, trnL-trnF, rps16	[18]
Rheum compactum	Leaves and roots	Dried	Yes	PCR, sequencing	trnL-trnF	[172]
Rheum hoatoense	Leaves and roots	Dried	Yes	PCR, sequencing	trnL-trnF	[172]
Rheum likiangense	Leaves and roots	Dried	Yes	PCR, sequencing	trnL-trnF	[172]
Rheum nanum	Leaves and roots	Dried	Yes	PCR, sequencing	trnL-trnF	[172]
Rheum officinale	Leaves and roots	Dried	Yes	PCR, sequencing	trnL-trnF	[172]
Rheum palmatum	Leaves and roots	Dried	Yes	PCR, sequencing	trnL-trnF	[172]
Rheum przewalskyi	Leaves and roots	Dried	Yes	PCR, sequencing	trnL-trnF	[172]
Rheum pumilum	Leaves and roots	Dried	Yes	PCR, sequencing	trnL-trnF	[172]
Rheum reticulatum	Leaves and roots	Dried	Yes	PCR, sequencing	trnL-trnF	[172]
Rheum sublanceolatum	Leaves and roots	Dried	Yes	PCR, sequencing	trnL-trnF	[172]
Rheum tanguticum	Leaves and roots	Dried	Yes	PCR, sequencing	trnL-trnF	[172]
Rheum undulatum	Leaves and roots	Dried	Yes	PCR, sequencing	trnL-trnF	[172]
Rheum wittrockii	Leaves and roots	Dried	Yes	PCR, sequencing	trnL-trnF	[172]
Rhodiola chrysanthemifolia	Leaves	Fresh		ISSR-PCR	N/A	[173]
Rhododendrom molle				PCR, sequencing; microarray (silicon)	5S gene spacer	[44]
Salvia bowleyana				PCR, sequencing	ITS	[174]
Salvia chinensis				PCR, sequencing	ITS	[174]
Salvia miltiorrhiza				PCR, sequencing	ITS	[174]
Salvia miltiorrhiza f. alba				PCR, sequencing	ITS	[174]
Salvia plebeia				PCR, sequencing	ITS	[174]
Salvia przewalskii				PCR, sequencing	ITS	[174]
Salvia substonifara				PCR, sequencing	ITS	[174]
Salvia trijuga				PCR, sequencing	ITS	[174]
Salvia yunnanensis				PCR, sequencing	ITS	[174]
Scutellaria altissima	Leaves	Fresh	Yes	PCR, sequencing	rpl16; rpl16-rpl14	[175]
Scutellaria baicalensis	Leaves	Fresh	Yes	PCR, sequencing	rpl16; rpl16-rpl14	[175]
				RAPD	N/A	[176]
	Leaves		Yes	RAPD	N/A	[177]
Scutellaria gelericulata	Leaves	Fresh	Yes	PCR, sequencing	rpl16; rpl16-rpl14	[175]
	Leaves		Yes	RAPD	N/A	[177]
Scutellaria incana	Leaves	Fresh	Yes	PCR, sequencing	rpl16; rpl16-rpl14	[175]
Scutellaria indica	Leaves	Fresh	Yes	PCR, sequencing	rpl16; rpl16-rpl14	[175]
Scutellaria laterifloria	Leaves	Fresh	Yes	PCR, sequencing	rpl16; rpl16-rpl14	[175]
	Leaves		Yes	RAPD	N/A	[177]
Sinopodophyllum hexandrum	Leaves	Dried	Yes	PCR, RFLP	trnT-trnL; trnD-trnT	[151]
Stellera chamaejasme				PCR, sequencing; microarray (silicon)	trnL	[44]
Strychnos nux-vomica	Leaves	Fresh	Yes	PCR, sequencing; microarray (silicon)	5S gene spacer	[44]
Swertia angustifolia			Yes	PCR, sequencing; allele-specific diagnostic PCR	rpl16; ITS	[156]
Swertia chirayita			Yes	PCR, sequencing; allele-specific diagnostic PCR	rpl16; ITS	[156]

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Plant (scientific name)	Part	Condition	Voucher	Method	Gene	Ref
Swertia dichotoma			Yes	PCR, sequencing; allele-specific diagnostic PCR	rpl16; ITS	[156]
Swertia erythrosticta			Yes	PCR, sequencing; allele-specific diagnostic PCR	rpl16; ITS	[156]
Swertia luquanensis			Yes	PCR, sequencing; allele-specific diagnostic PCR	rpl16; ITS	[156]
Swertia macrosperma			Yes	PCR, sequencing; allele-specific diagnostic PCR	rpl16; ITS	[156]
Swertia mileensis			Yes	PCR, sequencing; allele-specific diagnostic PCR	rpl16; ITS	[156]
Swertia mussotii			Yes	PCR, sequencing; allele-specific diagnostic PCR	rpl16; ITS	[156]
Swertia prsewalskii			Yes	PCR, sequencing; allele-specific diagnostic PCR	rpl16; ITS	[156]
Swertia punicea			Yes	PCR, sequencing; allele-specific diagnostic PCR	rpl16; ITS	[156]
Swertia tetraptera			Yes	PCR, sequencing; allele-specific diagnostic PCR	rpl16; ITS	[156]
Talinum paniculatum	Roots	Fresh, dried	Yes	AP-PCR; RAPD	N/A	[71]
Trifolium pratense	Leaves; dried ground material	Fresh, dried		PCR, sequencing; RFLP	ITS	[161]
Thymus vulgaris	Leaves			RAPD	N/A	[178]
Typhonium divaricatum	Leaves	Fresh	Yes	PCR, sequencing; microarray (silicon)	5S gene spacer	[44]
Typhonium flagelliforme	Leaves	Fresh		RAPD	N/A	[179]
Typhonium giganteum	Leaves	Fresh	Yes	PCR, sequencing; microarray (silicon)	5S gene spacer	[44]
Typhonium roxburghii	Leaves	Fresh		RAPD	N/A	[179]
Typhonium trilobatum	Leaves	Fresh		RAPD	N/A	[179]
Vitex rotundifolia	Fruits and leaves	Fresh	Yes	ISSR-PCR	N/A	[19]
Welwitschia mirabilis	Aerial parts	Fresh	Yes	PCR, sequencing	psbA-trnH	[85]

as input [20]. Although the overall results were similar, these authors found that the 5S rRNA spacer exhibited more sequence variation than either the ITS or 18S coding sequences and therefore proved best suited for the phylogenetic analysis of the *Astagalus* taxa examined [20]. Although the levels of isoflavonoids and astragalosides in each of 10 *Astragalus* taxa collected from 28 different regions exhibited variation, the phytochemical profiles did not allow for species level differentiation [110].

Conclusions

A large number of molecular techniques have been used to authenticate medicinal plants based on species-specific variations in the sequences of various chloroplast and nuclear DNA regions. Using PCR-based methods, species identification has been achieved using DNA that was isolated from fresh and dried plant parts, plant extracts, processed herbal drugs, as well as finished products such as herbal teas, tablets and capsules. Genomic fingerprinting can differentiate between individuals, species and populations and has proven useful for the characterization of sample homogeneity and detection of adulterants.

DNA-based authentication of medicinal plants is a work in progress that offers powerful new tools and entry points for measures aimed at quality control and quality assurance in medical plant research as well as the production, clinical use, and forensic examination of herbal medicines. For example, genome-based methods can be useful in quickly and efficiently pinpointing adulterated or misidentified raw materials, which can then be discarded without further need for time- and resource-consuming morphological, physical and phytochemical examinations. However, DNA-based species identification alone will rarely be sufficient for quality control and assurance because, as living organisms, plants are the product of both the genome and the environment. Although both qualitative and quantitative properties of plant metabolic pathways are largely predetermined genetically, overall metabolic activity is strongly influenced by the environment. Moreover, metabolites are often distributed unequally in different parts of the plant such as roots, stems or leaves, for example. Considering the important role that the chemical metabolites are thought to play in mediating the pharmacologic effects of herbal medicines [111], [112], the importance of extensive and standardized phytochemical characterization of medicinal plants by chromatographic and spectroscopic methods will continue to grow [113].

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