Genome-Based Approaches to the Authentication of Medicinal Plants

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Key words
- Medicinal plants
- traditional Chinese medicine
- authentication
- DNA fingerprinting
- genotyping
- plant barcoding

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-
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 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>
<thead>
<tr>
<th>Name</th>
<th>Acronym</th>
<th>Explanation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polymerase chain reaction</td>
<td>PCR</td>
<td>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].</td>
</tr>
<tr>
<td>Allele-specific diagnostic PCR</td>
<td></td>
<td>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.</td>
</tr>
<tr>
<td>Amplification refractory mutation system</td>
<td>ARMS</td>
<td>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].</td>
</tr>
<tr>
<td>Amplified fragmented length polymorphism</td>
<td>AFLP</td>
<td>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].</td>
</tr>
<tr>
<td>Arbitrarily primed PCR</td>
<td>AP-PCR</td>
<td>Similar to RAPD but PCR is performed using sets of two longer primers (&gt;18 nucleotides) of arbitrary sequence.</td>
</tr>
<tr>
<td>Direct amplification of length polymorphism</td>
<td>DALP</td>
<td>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].</td>
</tr>
<tr>
<td>Multiplex PCR</td>
<td></td>
<td>PCR with multiple sets of forward and reverse primers in the same reaction resulting in parallel amplification [116].</td>
</tr>
<tr>
<td>PCR-selective restriction</td>
<td>PCR-SR</td>
<td>PCR amplicons obtained with gene specific primers are cut with restriction enzymes and analyzed by gel electrophoresis [121].</td>
</tr>
<tr>
<td>Randomly amplified polymorphic DNA</td>
<td>RAPD</td>
<td>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].</td>
</tr>
<tr>
<td>Sequence characterized amplified region</td>
<td>SCAR</td>
<td>Distinct amplicons obtained by RAPD are sequenced and amplicon specific primers are designed for use in PCR [122].</td>
</tr>
<tr>
<td>Restriction length polymorphism</td>
<td>RFLP</td>
<td>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. A DNA microarray, 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.</td>
</tr>
<tr>
<td>DNA sequencing</td>
<td></td>
<td>DNA sequencing is now almost exclusively performed using cycle sequencing, which is conducted using a heat stable DNA polymerase and fluorescently labeled deoxyribonucleotides in a thermocycler. 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.</td>
</tr>
<tr>
<td>Inter simple sequence repeat-anchored PCR</td>
<td>ISSR-PCR</td>
<td>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].</td>
</tr>
<tr>
<td>Multiplex amplification refractory mutation system</td>
<td>MARMS</td>
<td>Multiplex PCR using a common primer and multiple mutation specific primers as used in ARMS [126].</td>
</tr>
<tr>
<td>Simple sequence repeat polymorphism</td>
<td>SSR</td>
<td>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., CACA-CA 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].</td>
</tr>
</tbody>
</table>
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,
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, ITS and 26S rRNA genes [58], [59], [60] and the intergenic spacers that separate multiple repeated copies of the nuclear SS 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, rp0B, and rpoC1, the trnL intron and intergenic spacers between the trnC-trnD, trnL-trnF, trnH-psbA, and psbK-psbKl 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., leaf 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 SS 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 SS 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
<table>
<thead>
<tr>
<th>Plant (scientific name)</th>
<th>Part</th>
<th>Condition</th>
<th>Voucher</th>
<th>Method</th>
<th>Gene</th>
<th>Ref</th>
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</thead>
<tbody>
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<td>Aconitum carmichaeli</td>
<td>Leaves</td>
<td>Fresh</td>
<td>Yes</td>
<td>PCR, sequencing; microarray (silicon)</td>
<td>5S gene spacer</td>
<td>[44]</td>
</tr>
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<td>AFLP</td>
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<td>[134]</td>
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<td></td>
<td>AFLP</td>
<td>N/A</td>
<td>[134]</td>
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<td>Actaea cordifolia</td>
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<td></td>
<td>AFLP</td>
<td>N/A</td>
<td>[134]</td>
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<tr>
<td>Actaea podocarpa</td>
<td>Leaves</td>
<td></td>
<td></td>
<td>AFLP</td>
<td>N/A</td>
<td>[134]</td>
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<td>Actaea pachypoda</td>
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<td></td>
<td>AFLP</td>
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<td>[134]</td>
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<tr>
<td>Adenophora hunenensis</td>
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<td>Yes</td>
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<tr>
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<td></td>
<td>Yes</td>
<td>PCR, sequencing</td>
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<tr>
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<td></td>
<td>Yes</td>
<td>PCR, sequencing</td>
<td>5S gene spacer</td>
<td>[135]</td>
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<td>Agastache foeniculum</td>
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<td>PCR, sequencing</td>
<td>18S rRNA; matK</td>
<td>[136]</td>
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<td>Agastache rugosa</td>
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<td>PCR, sequencing</td>
<td>18S rRNA; matK</td>
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<td>Alisma canaliculatum</td>
<td>Rhizome</td>
<td>Dried</td>
<td>Yes</td>
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<td>ITS</td>
<td>[137]</td>
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<td>Alisma gracilis</td>
<td>Rhizome</td>
<td>Dried</td>
<td>Yes</td>
<td>PCR, sequencing; RFLP; ARMS</td>
<td>ITS</td>
<td>[137]</td>
</tr>
<tr>
<td>Alisma lanceolatium</td>
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<td>Yes</td>
<td>PCR, sequencing; RFLP; ARMS</td>
<td>ITS</td>
<td>[137]</td>
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<tr>
<td>Alisma nanum</td>
<td>Rhizome</td>
<td>Dried</td>
<td>Yes</td>
<td>PCR, sequencing; RFLP; ARMS</td>
<td>ITS</td>
<td>[137]</td>
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<tr>
<td>Alisma orientale</td>
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<td>Dried</td>
<td>Yes</td>
<td>PCR, sequencing; RFLP; ARMS</td>
<td>ITS</td>
<td>[137]</td>
</tr>
<tr>
<td>Alisma plantago-aquatica</td>
<td>Rhizome</td>
<td>Dried</td>
<td>Yes</td>
<td>PCR, sequencing; RFLP; ARMS</td>
<td>ITS</td>
<td>[137]</td>
</tr>
<tr>
<td>Alocasia macrorrhiza</td>
<td>Leaves</td>
<td>Fresh</td>
<td>Yes</td>
<td>PCR, sequencing; microarray (silicon)</td>
<td>5S gene spacer</td>
<td>[44]</td>
</tr>
<tr>
<td>Angelica acutiloba</td>
<td>Dried</td>
<td></td>
<td>Yes</td>
<td>PCR, sequencing</td>
<td>5S gene spacer</td>
<td>[107]</td>
</tr>
<tr>
<td>Angelica acutiloba var. acutiloba</td>
<td>Leaves</td>
<td>Fresh</td>
<td>Yes</td>
<td>PCR, sequencing</td>
<td>5S gene spacer</td>
<td>[138]</td>
</tr>
<tr>
<td>Angelica acutiloba var. iwatensis</td>
<td>Leaves</td>
<td>Fresh</td>
<td>Yes</td>
<td>PCR, sequencing</td>
<td>5S gene spacer</td>
<td>[138]</td>
</tr>
<tr>
<td>Angelica acutiloba var. sugijame</td>
<td>Leaves</td>
<td>Fresh</td>
<td>Yes</td>
<td>PCR, sequencing</td>
<td>5S gene spacer</td>
<td>[138]</td>
</tr>
<tr>
<td>Angelica acutiloba var. Sugiyame</td>
<td>Leaves</td>
<td>Fresh</td>
<td>Yes</td>
<td>RAPD; RFLP</td>
<td>N/A</td>
<td>[139]</td>
</tr>
<tr>
<td>Angelica gigas</td>
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<td></td>
<td>Yes</td>
<td>PCR, sequencing</td>
<td>5S gene spacer</td>
<td>[107]</td>
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<tr>
<td>Angelica sinensis</td>
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<td></td>
<td>Yes</td>
<td>PCR, sequencing</td>
<td>5S gene spacer</td>
<td>[107]</td>
</tr>
<tr>
<td>Angelica sinensis</td>
<td>Root</td>
<td>Dried</td>
<td></td>
<td>RAPD; RFLP</td>
<td>N/A</td>
<td>[139]</td>
</tr>
<tr>
<td>Aralia elata</td>
<td></td>
<td></td>
<td>Yes</td>
<td>PCR, sequencing</td>
<td>ITS; trnC-trnD</td>
<td>[69]</td>
</tr>
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<td>Aralia franchetii</td>
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<td></td>
<td>Yes</td>
<td>PCR, sequencing</td>
<td>ITS; trnC-trnD</td>
<td>[69]</td>
</tr>
<tr>
<td>Arisaema heterophyllum</td>
<td>Leaves</td>
<td>Fresh</td>
<td>Yes</td>
<td>PCR, sequencing; PCR-SR</td>
<td>Mannose-binding lectin</td>
<td>[121]</td>
</tr>
<tr>
<td>Artemisia aponica</td>
<td>Leaves</td>
<td>Fresh</td>
<td></td>
<td>PCR, sequencing; SCAR</td>
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<tr>
<td>Artemisia argyi</td>
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<td>Fresh</td>
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<td>PCR, sequencing; SCAR</td>
<td>N/A</td>
<td>[140]</td>
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<tr>
<td>Artemisia capillaries</td>
<td>Leaves</td>
<td>Fresh</td>
<td></td>
<td>PCR, sequencing; SCAR</td>
<td>N/A</td>
<td>[140]</td>
</tr>
<tr>
<td>Artemisia iwayomogi</td>
<td>Leaves</td>
<td>Fresh</td>
<td></td>
<td>PCR, sequencing; SCAR</td>
<td>N/A</td>
<td>[140]</td>
</tr>
<tr>
<td>Artemisia keiskeana</td>
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<td>Fresh</td>
<td></td>
<td>PCR, sequencing; SCAR</td>
<td>N/A</td>
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<tr>
<td>Artemisia princeo</td>
<td>Leaves</td>
<td>Fresh</td>
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<td>PCR, sequencing; SCAR</td>
<td>N/A</td>
<td>[140]</td>
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Sucher NJ, Carles MC. Genome-Based Approaches to... Planta Med 2008; 74: 603–623
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Sucher NJ, Carles MC. Genome-Based Approaches to... Planta Med 2008; 74: 603–623
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<td>Yes</td>
<td>PCR, sequencing; allele-specific diagnostic PCR</td>
<td>rpl16; ITS</td>
<td>[156]</td>
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<td><em>Gnetum gnemon</em></td>
<td>Aerial parts</td>
<td>Fresh, crude drug</td>
<td>Yes</td>
<td>PCR, sequencing</td>
<td>ITS; psbA-trnH</td>
<td>[85]</td>
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<td><em>Gnetum lepostachyum</em></td>
<td>Stems</td>
<td>Fresh</td>
<td>Yes</td>
<td>PCR, sequencing</td>
<td>ITS; trnL; trnL-trnF</td>
<td>[84]</td>
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<td><em>Halenia elliptica</em></td>
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<td></td>
<td></td>
<td>PCR, sequencing</td>
<td>ITS</td>
<td>[156]</td>
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<td><em>Hedysarum polybotris</em></td>
<td>Leaves, roots</td>
<td>Fresh, crude drug</td>
<td>Yes</td>
<td>PCR, sequencing</td>
<td>5S gene spacer</td>
<td>[104]</td>
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<td><em>Hemerocallis citrina</em></td>
<td>Leaf</td>
<td>Fresh, crude drug</td>
<td>Yes</td>
<td>PCR, sequencing</td>
<td>5S gene spacer</td>
<td>[147]</td>
</tr>
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<td><em>Humulus hops</em></td>
<td>Leaves, stems, flowering heads</td>
<td>Fresh, crude drug</td>
<td>Yes</td>
<td>PCR, sequencing</td>
<td>RAPD</td>
<td>N/A</td>
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<td><em>Hyoscyamus niger</em></td>
<td>Leaves</td>
<td>Fresh</td>
<td>Yes</td>
<td>PCR, sequencing; microarray (silicon)</td>
<td>5S gene spacer</td>
<td>[44]</td>
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<td><em>Lamium amplexicaule</em></td>
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<td></td>
<td>PCR, sequencing</td>
<td>ITS</td>
<td>[158]</td>
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<td><em>Leonurus chaotauroides</em></td>
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<td></td>
<td></td>
<td>PCR, sequencing</td>
<td>ITS</td>
<td>[158]</td>
</tr>
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<td><em>Leonurus heterophyllus</em></td>
<td></td>
<td></td>
<td></td>
<td>PCR, sequencing</td>
<td>ITS</td>
<td>[158]</td>
</tr>
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<td><em>Leonurus pseudomacranthus</em></td>
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<td></td>
<td></td>
<td>PCR, sequencing</td>
<td>ITS</td>
<td>[158]</td>
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<td><em>Leonurus sibiricus</em></td>
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<td>PCR, sequencing</td>
<td>ITS</td>
<td>[158]</td>
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<td><em>Ligularia dentata</em></td>
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<td>Yes</td>
<td>PCR, sequencing</td>
<td>5S gene spacer</td>
<td>[159]</td>
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<td><em>Ligularia kniazensis</em></td>
<td></td>
<td>Yes</td>
<td>PCR, sequencing</td>
<td>5S gene spacer</td>
<td>[159]</td>
<td></td>
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<td><em>Ligularia lankongensis</em></td>
<td></td>
<td>Yes</td>
<td>PCR, sequencing</td>
<td>5S gene spacer</td>
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<td><em>Ligularia lapathifolia</em></td>
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<td>Yes</td>
<td>PCR, sequencing</td>
<td>5S gene spacer</td>
<td>[159]</td>
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<td><em>Ligularia narynensis</em></td>
<td></td>
<td>Yes</td>
<td>PCR, sequencing</td>
<td>5S gene spacer</td>
<td>[159]</td>
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<tr>
<td><em>Ligularia nelumbifolia</em></td>
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<td>Yes</td>
<td>PCR, sequencing</td>
<td>5S gene spacer</td>
<td>[159]</td>
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<td><em>Ligularia pleurocalulis</em></td>
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<td>Yes</td>
<td>PCR, sequencing</td>
<td>5S gene spacer</td>
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<td><em>Ligularia przewalskii</em></td>
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<td>Yes</td>
<td>PCR, sequencing</td>
<td>5S gene spacer</td>
<td>[159]</td>
<td></td>
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<td><em>Ligularia sagitta</em></td>
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<td>Yes</td>
<td>PCR, sequencing</td>
<td>5S gene spacer</td>
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<td><em>Ligularia subspicata</em></td>
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<td>Yes</td>
<td>PCR, sequencing</td>
<td>5S gene spacer</td>
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<td><em>Ligularia tangolensis</em></td>
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<td>Yes</td>
<td>PCR, sequencing</td>
<td>5S gene spacer</td>
<td>[159]</td>
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<td><em>Ligularia virgaurea</em></td>
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<td>Yes</td>
<td>PCR, sequencing</td>
<td>5S gene spacer</td>
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<td><em>Lomatogonium oreocharis</em></td>
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<td>Yes</td>
<td>PCR, sequencing; allele-specific diagnostic PCR</td>
<td>rpl16; ITS</td>
<td>[156]</td>
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<tr>
<td><em>Lycium barbarum</em></td>
<td>Fruit</td>
<td>Dried</td>
<td>Yes</td>
<td>RAPD</td>
<td>N/A</td>
<td>[160]</td>
</tr>
<tr>
<td><em>Lycium barbarum cv. &quot;Tianjoenese&quot;</em></td>
<td>Fruit</td>
<td>Dried</td>
<td>Yes</td>
<td>RAPD</td>
<td>N/A</td>
<td>[160]</td>
</tr>
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<td><em>Lycium barbarum var. aranticarpum</em></td>
<td>Fruit</td>
<td>Dried</td>
<td>Yes</td>
<td>RAPD</td>
<td>N/A</td>
<td>[160]</td>
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<td><em>Lycium barbarum var. potaninii</em></td>
<td>Fruit</td>
<td>Dried</td>
<td>Yes</td>
<td>RAPD</td>
<td>N/A</td>
<td>[160]</td>
</tr>
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<td><em>Lycium chinense</em></td>
<td>Fruit</td>
<td>Dried</td>
<td>Yes</td>
<td>RAPD</td>
<td>N/A</td>
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<td><em>Lycium dasy Stemsum var. rubricoaulium</em></td>
<td>Fruit</td>
<td>Dried</td>
<td>Yes</td>
<td>RAPD</td>
<td>N/A</td>
<td>[160]</td>
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<td><em>Lycium ruthenicum</em></td>
<td>Fruit</td>
<td>Dried</td>
<td>Yes</td>
<td>RAPD</td>
<td>N/A</td>
<td>[160]</td>
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<td><em>Lycium truncatum</em></td>
<td>Fruit</td>
<td>Dried</td>
<td>Yes</td>
<td>RAPD</td>
<td>N/A</td>
<td>[160]</td>
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<td><em>Medicago sativa</em></td>
<td>Leaves; dried ground material</td>
<td>Fresh, dried</td>
<td>Yes</td>
<td>PCR, sequencing; RFLP</td>
<td>ITS</td>
<td>[161]</td>
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<td><em>Mirablis jalapa</em></td>
<td>Roots</td>
<td>Fresh, dried</td>
<td>Yes</td>
<td>AP-PCR; RAPD</td>
<td>N/A</td>
<td>[71]</td>
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<td><em>Nandina domestica</em></td>
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<td></td>
<td></td>
<td>PCR, sequencing</td>
<td>5S gene spacer</td>
<td>[154]</td>
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<td><em>Panax assamicus</em></td>
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<td></td>
<td>PCR, sequencing</td>
<td>ITS; trnC-trnD</td>
<td>[69]</td>
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<td><em>Panax bipinnatifidus var. angustifolius</em></td>
<td></td>
<td></td>
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<td>PCR, sequencing</td>
<td>ITS; trnC-trnD</td>
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<td>Part</td>
<td>Condition</td>
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<td>Gene</td>
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<td><em>Panax bipinnatifidus</em> var. <em>bipinnatifidus</em></td>
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<td></td>
<td>Yes</td>
<td>PCR, sequencing</td>
<td>ITS; trnC-trnD</td>
<td>[69]</td>
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<td><em>Panax eleganterior</em></td>
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<td>Yes</td>
<td>PCR, sequencing</td>
<td>ITS; trnC-trnD</td>
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<td><em>Panax ginseng</em></td>
<td>Roots</td>
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<td>Yes</td>
<td>PCR, sequencing</td>
<td>ITS; trnC-trnD</td>
<td>[69]</td>
</tr>
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<td></td>
<td>Roots</td>
<td>Fresh, dried</td>
<td></td>
<td>AP-PCR; RAPD</td>
<td>N/A</td>
<td>[71]</td>
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<td></td>
<td>Roots</td>
<td>Fresh, dried</td>
<td>Yes</td>
<td>RAPD, sequencing; SCAR</td>
<td>N/A</td>
<td>[162]</td>
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<td>Roots</td>
<td>Fresh, dried</td>
<td>Yes</td>
<td>RAPD, DALP, sequencing</td>
<td>N/A</td>
<td>[66]</td>
</tr>
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<td></td>
<td>Leaves, roots</td>
<td>Fresh, crude drug</td>
<td>Yes</td>
<td>PCR</td>
<td>SSR</td>
<td>[163]</td>
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<td>RAPD</td>
<td>N/A</td>
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<td>Crude drug</td>
<td>Yes</td>
<td>PCR, sequencing</td>
<td>ITS; trnC-trnD</td>
<td>[69]</td>
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<td><em>Panax japonicus</em></td>
<td>Leaves, roots</td>
<td>Fresh, crude drug</td>
<td>Yes</td>
<td>MARMs</td>
<td>tmK, 18S rRNA</td>
<td>[74]</td>
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<td>Crude drug</td>
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<td></td>
<td>PCR</td>
<td>N/A</td>
<td>[66]</td>
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<td><em>Panax major</em></td>
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<td>Yes</td>
<td>PCR, sequencing</td>
<td>ITS; trnC-trnD</td>
<td>[69]</td>
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<td><em>Panax notoginseng</em></td>
<td>Roots</td>
<td>Fresh, dried</td>
<td>Yes</td>
<td>AP-PCR; RAPD</td>
<td>N/A</td>
<td>[71]</td>
</tr>
<tr>
<td></td>
<td>Leaves, roots</td>
<td>Fresh, crude drug</td>
<td>Yes</td>
<td>MARMs</td>
<td>tmK, 18S rRNA</td>
<td>[74]</td>
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<td>Crude drug</td>
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<td></td>
<td>RAPD</td>
<td>N/A</td>
<td>[72]</td>
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<td>Yes</td>
<td>PCR, sequencing</td>
<td>ITS; trnC-trnD</td>
<td>[69]</td>
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<td>AfLPl, PCR, sequencing</td>
<td>ITS 2</td>
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<td><em>Panax omeiensis</em></td>
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<td>Yes</td>
<td>PCR, sequencing</td>
<td>ITS; trnC-trnD</td>
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<td><em>Panax pseudoginseng</em></td>
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<td>Yes</td>
<td>PCR, sequencing</td>
<td>ITS; trnC-trnD</td>
<td>[69]</td>
</tr>
<tr>
<td><em>Panax quinquefolium</em></td>
<td>Roots</td>
<td></td>
<td>Yes</td>
<td>AP-PCR</td>
<td>N/A</td>
<td>[71]</td>
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<td></td>
<td>Roots</td>
<td>Fresh, dried</td>
<td>Yes</td>
<td>AP-PCR; RAPD</td>
<td>N/A</td>
<td>[71]</td>
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<td></td>
<td>Roots</td>
<td>Fresh, dried</td>
<td>Yes</td>
<td>RAPD, sequencing; SCAR</td>
<td>N/A</td>
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<td>Roots</td>
<td>Fresh, dried</td>
<td>Yes</td>
<td>RAPD, DALP, sequencing</td>
<td>N/A</td>
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<td></td>
<td>Leaves, roots</td>
<td>Fresh, crude drug</td>
<td>Yes</td>
<td>PCR</td>
<td>MARMs</td>
<td>tmK, 18S rRNA</td>
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<td><em>Panax quinquefolius</em></td>
<td>Crude drug</td>
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<td>PCR</td>
<td>Microsatellite marker</td>
<td>N/A</td>
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<td>RAPD</td>
<td>N/A</td>
<td>[72]</td>
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<td><em>Panax shangianus</em></td>
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<td>Yes</td>
<td>PCR, sequencing</td>
<td>ITS; trnC-trnD</td>
<td>[69]</td>
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<td><em>Panax sinensis</em></td>
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<td>Yes</td>
<td>PCR, sequencing</td>
<td>ITS; trnC-trnD</td>
<td>[69]</td>
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<td><em>Panax stipulatus</em></td>
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<td>Yes</td>
<td>PCR, sequencing</td>
<td>ITS; trnC-trnD</td>
<td>[69]</td>
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<td><em>Panax trifolius</em></td>
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<td>Yes</td>
<td>PCR, sequencing</td>
<td>ITS; trnC-trnD</td>
<td>[69]</td>
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<td><em>Panax variabilis</em></td>
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<td>Yes</td>
<td>PCR, sequencing</td>
<td>ITS; trnC-trnD</td>
<td>[69]</td>
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<td><em>Panax vietnamensis</em></td>
<td>Leaves, roots</td>
<td>Fresh, crude drug</td>
<td>Yes</td>
<td>PCR</td>
<td>MARMs</td>
<td>tmK, 18S rRNA</td>
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<td>Crude drug</td>
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<td>PCR</td>
<td>N/A</td>
<td>[66]</td>
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<td><em>Panax wangerianus</em></td>
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<td>Yes</td>
<td>PCR, sequencing</td>
<td>ITS; trnC-trnD</td>
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<td><em>Panax zingiberensis</em></td>
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<td>Yes</td>
<td>PCR, sequencing</td>
<td>ITS; trnC-trnD</td>
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<td><em>Perilla frutescens</em></td>
<td></td>
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<td>Yes</td>
<td>PCR, sequencing</td>
<td>ITS</td>
<td>[164]</td>
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<td><em>Perilla frutescens var. arguta</em></td>
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<td>Yes</td>
<td>PCR, sequencing</td>
<td>ITS</td>
<td>[164]</td>
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<td>PCR</td>
<td>N/A</td>
<td>[66]</td>
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<td><em>Perilla frutescens var. auriculato-dentata</em></td>
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<td>PCR, sequencing</td>
<td>ITS</td>
<td>[164]</td>
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<tr>
<td><em>Perilla frutescens var. crispa</em></td>
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<td></td>
<td>PCR, sequencing</td>
<td>ITS</td>
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<tr>
<td><em>Pholidota cantonensis</em></td>
<td>Stems</td>
<td>Fresh</td>
<td>Yes</td>
<td>PCR, sequencing</td>
<td>ITS</td>
<td>[94]</td>
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<td><em>Phyllanthus amarus</em></td>
<td></td>
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<td>Yes</td>
<td>PCR, sequencing; multiplex PCR</td>
<td>ITS; atpB; rbcL</td>
<td>[165]</td>
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<td><em>Phyllanthus arenarius</em></td>
<td></td>
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<td>Yes</td>
<td>PCR, sequencing; multiplex PCR</td>
<td>ITS; atpB; rbcL</td>
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<td><em>Phyllanthus calycinus</em></td>
<td></td>
<td></td>
<td>Yes</td>
<td>PCR, sequencing; multiplex PCR</td>
<td>ITS; atpB; rbcL</td>
<td>[165]</td>
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<td><em>Phyllanthus clakei</em></td>
<td></td>
<td></td>
<td>Yes</td>
<td>PCR, sequencing; multiplex PCR</td>
<td>ITS; atpB; rbcL</td>
<td>[165]</td>
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<td><em>Phyllanthus cochinchnensis</em></td>
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<td>Yes</td>
<td>PCR, sequencing; multiplex PCR</td>
<td>ITS; atpB; rbcL</td>
<td>[165]</td>
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<td><em>Phyllanthus distichus</em></td>
<td>Leaves</td>
<td>Fresh</td>
<td>Yes</td>
<td>PCR, sequencing; multiplex PCR</td>
<td>ITS; atpB; rbcL</td>
<td>[165]</td>
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<td>RAPD, sequencing SCAR</td>
<td>N/A</td>
<td>[166]</td>
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<td><em>Phyllanthus emblica</em> (= <em>Emblica officinalis</em>)</td>
<td>Leaves</td>
<td>Fresh and dried</td>
<td>Yes</td>
<td>PCR, sequencing; multiplex PCR</td>
<td>ITS; atpB; rbcL</td>
<td>[165]</td>
</tr>
<tr>
<td><em>Phyllanthus emblica</em> (= <em>Emblica officinalis</em>)</td>
<td>Leaves</td>
<td>Fresh and dried</td>
<td>Yes</td>
<td>PCR, sequencing; multiplex PCR</td>
<td>ITS; atpB; rbcL</td>
<td>[165]</td>
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<td><em>Phyllanthus flexuosus</em></td>
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<td>Yes</td>
<td>PCR, sequencing; multiplex PCR</td>
<td>ITS; atpB; rbcL</td>
<td>[165]</td>
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<td>Plant (scientific name)</td>
<td>Part</td>
<td>Condition</td>
<td>Voucher</td>
<td>Method</td>
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<td>Ref</td>
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<td><em>Phyllanthus glaucus</em></td>
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Sucher NJ, Carles MC. Genome-Based Approaches to... Planta Med 2008; 74: 603–623.
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 *Astragalus* 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 medicinal 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].

**References**


<table>
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<td>Yes</td>
<td>PCR, sequencing; allele-specific diagnostic PCR</td>
<td>rpl16; ITS</td>
<td>[156]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Swertia tetraptera</td>
<td>Yes</td>
<td>PCR, sequencing; allele-specific diagnostic PCR</td>
<td>rpl16; ITS</td>
<td>[156]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Talinum paniculatum</td>
<td>Roots</td>
<td>Fresh, dried</td>
<td>Yes</td>
<td>AP-PCR; RAPD PCR, sequencing; RFLP</td>
<td>N/A</td>
<td>[71]</td>
</tr>
<tr>
<td>Trifolium pratense</td>
<td>Leaves; dried material</td>
<td>Fresh, dried</td>
<td>Yes</td>
<td>N/A</td>
<td>[161]</td>
<td></td>
</tr>
<tr>
<td>Thymus vulgaris</td>
<td>Leaves</td>
<td>Fresh</td>
<td>Yes</td>
<td>RAPD PCR, sequencing; microarray (silicon)</td>
<td>N/A</td>
<td>[178]</td>
</tr>
<tr>
<td>Typhonium divaricatum</td>
<td>Leaves</td>
<td>Fresh</td>
<td>Yes</td>
<td>RAPD</td>
<td>N/A</td>
<td>[179]</td>
</tr>
<tr>
<td>Typhonium flagelliforme</td>
<td>Leaves</td>
<td>Fresh</td>
<td>Yes</td>
<td>PCR, sequencing; microarray (silicon)</td>
<td>SS gene spacer</td>
<td>[44]</td>
</tr>
<tr>
<td>Typhonium giganteum</td>
<td>Leaves</td>
<td>Fresh</td>
<td>Yes</td>
<td>PCR, sequencing; microarray (silicon)</td>
<td>SS gene spacer</td>
<td>[44]</td>
</tr>
<tr>
<td>Typhonium roxburghii</td>
<td>Leaves</td>
<td>Fresh</td>
<td>Yes</td>
<td>RAPD</td>
<td>N/A</td>
<td>[179]</td>
</tr>
<tr>
<td>Typhonium trilobaturn</td>
<td>Leaves</td>
<td>Fresh</td>
<td>Yes</td>
<td>RAPD</td>
<td>N/A</td>
<td>[179]</td>
</tr>
<tr>
<td>Vitex rotundifolia</td>
<td>Fruits and leaves</td>
<td>Fresh</td>
<td>Yes</td>
<td>ISSR-PCR</td>
<td>N/A</td>
<td>[19]</td>
</tr>
<tr>
<td>Welwitschia mirabilis</td>
<td>Aerial parts</td>
<td>Fresh</td>
<td>Yes</td>
<td>PCR, sequencing</td>
<td>psbA-trnH</td>
<td>[85]</td>
</tr>
</tbody>
</table>

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