Identification of botanicals using molecular biotechnology

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Abstract

The available information on the abundance of restorative plants on earth is incomplete, and the data regarding botanicals from various countries differ significantly. The substantial development of the worldwide natural botanical market is attributable to the expanding revenue of global drug companies trading herbal medicines. This essential type of traditional medical care is depended on by approx. 72–80% of individuals. Even though numerous restorative plants are readily used, they have never been subject to the same strict quality guidelines as conventional drugs. Nonetheless, it is vital to have specific organic, phytochemical, and molecular tools and methods for identifying restorative plant species so that traditional and novel plant products can be safely used in modern medicine. Molecular biotechnology approaches provide a reliable and accurate way to identify botanicals and can be used to ensure the safety and efficacy of plant-based products. This review explores various molecular biotechnology approaches and methods for identifying botanicals.

Key words: genomic DNA, herbal drugs, phytotechnology

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Introduction

Evidence on character and safety is of primary importance among different essential factors in the quality verification of plant-based medications. Character refers to botanical identity, authenticity and quality of the plant material used, which requires the avoidance of quality reduction and misrepresentation of herbal medications. Such factors include the presence of harmful constituents, low efficacy or anything else that is related to the fundamental claim that the plant has health-promoting properties.[1]

In the context of herbal medications, misrepresentation refers to any deceptive or misleading practices that can occur during the production, labeling, or marketing of herbal products. This can include various forms of misrepresentation, such as:

Substitution: The intentional or unintentional substitution of one plant species with another that may have different therapeutic properties or potential safety concerns. For example, using a cheaper or more readily available plant as a substitute for the desired plant species.

Adulteration: The addition of undeclared substances or contaminants to the herbal product, which can affect its quality, efficacy, or safety. Adulteration can occur by adding lower quality plant material, synthetic compounds, or other substances that are not disclosed on the product label.

False labeling: Providing incorrect or misleading information on the product label, such as inaccurate botanical names, misleading claims about the product's composition or therapeutic effects, or incorrect information about the geographical origin or processing methods.

The morphology (visible with a naked eye) and appearance of plant-based medications have been demonstrated in the literature, yet it should be considered that the validity of a medication cannot be ensured with certainty by identification of plants alone. Indeed, significant experience in pharmacognostics and the utilization of legitimate standardized drugs are also required.[2]

Scientists can distinguish the complicated ingredients of all basic molecular elements of a natural supplement using imaging modalities and automated high-performance liquid chromatography (HPLC) analysis. Indeed, well-defined HPLC methods using specific markers meet the prerequisites of legitimate science-based verification of natural medications, and fulfilling strict requirements of the International Council for Harmonization Rejection (ICHR) regarding potential misrepresentations and corruption of herbal medications for wellbeing reasons should likewise be possible employing such procedures.[3]

All plants in the world are assigned a genus and species name for portrayal and references in herbaria since the founding of binomial terminology by Carl von Linne in 1753, such as *Chamomilla* (genus), and *recutita* and *inodora* (species). Based on genetic, morphological and structural attributes, subspecies and substance races should also be discerned so that an alternate subjective and quantitative synthetic structure of constituents can be created.[4]

Consequently, we should reason that surveying the natural properties of herbal medications is a fundamental necessity and should be the primary aim before a plant can be formally included into an authoritative Pharmacopeia.[5]

DNA-based confirmation of natural plants

Recently, therapeutic plants have become popular in Western nations, where the desire to use natural remedies has developed and such use is now considered an option by doctors and patients. There has been evidence of increasing interest in herbal drugs (natural preparations) as they are easily available and can be generally utilized as medicines with possibly lesser side effects in comparison to synthetically derived drugs. As such, they are protective and do not cause any antagonistic effects. Nonetheless, the natural origin of herbs does not guarantee their safety. Some herbs may have intrinsic toxicity, and there is a risk of substitution, contamination, and adulteration of herbal products. Additionally, misidentification of plant species can occur, especially considering the wide variety of species used in herbal medicine. These factors can have potential adverse effects on the quality and safety of botanical products.[6]

It has been demonstrated that contamination or substitution of herbs has caused illness and even deaths. A case of neuropathy and encephalopathy was recorded after the administration of a preparation derived from the herb *Gentiana rigescens radix*. Further examination revealed that poisoning had occurred due to contamination by *Podophyllum emodi* or *Podophyllum hexandrum*, which contain the neurotoxin podophyllotoxin and were found growing underneath the original preparation. A few instances of renal injury related to *Stephania tetrandra* in a weight reduction formulation were caused by *Aristolochia fangchi*, which contains the highly poisonous aristolochic acid, a nephrotoxin that causes urethral cancer, among others. In this case, the confusion arose from the similarity of the 2 plants in Chinese nomenclature.[7]

Consequently, to guarantee the quality and efficacy of natural medications, and ensure the wellbeing of the patients, identifying the plant species is essential. However, distinguishing natural components, which regularly comprise dried or prepared parts, is by and large troublesome due to the change of characteristics through drying, particularly when a herb has more than 1 name or when a common name is used for more than 1 herb.[8]

Validation of natural material on the DNA level

Genetics are thought to provide unwavering quality in the verification of natural materials at the DNA level due

to the advances in molecular biotechnology and plant genetics. Moreover, the use of DNA-based molecular marker methods for understanding species of therapeutic plants is still expanding. Such improvements are particularly helpful for identifying species that are regularly confused or adulterated with other plants, and for species that are morphologically or phytochemically indistinguishable.[9]

The DNA is a remarkably stable macromolecule that is not influenced by external factors, meaning that it can be recovered from fresh, dried and prepared natural materials. In contrast, substance fingerprinting is unequivocally influenced by the sample age, physiological conditions, environmental impact development region, reaping period, drying, and storage conditions. Moreover, the DNA marker particles are not tissue-specific and, in this way, can be distinguished at all phases of organic growth. Also, a modest quantity of a sample is adequate for examination.[10] Table 1 presents a comparison of DNA profiling techniques for herbal authentication and quality control.

Types of DNA markers utilized in plant genome analysis

The DNA-based molecular strategies that have been utilized to assess DNA polymorphisms for plant taxa verification include hybridization polymerase chain reaction (PCR) and sequencing techniques. Currently, multi-locus sequence analysis (MLSA) is frequently used in phylogenetic investigations and has demonstrated its discriminatory ability. In addition, recent improvements in the sensitivity of high-throughput DNA microarrays have allowed for the definitive identification of taxon on a larger scale.[11]

Random amplified polymorphic DNA

Random amplified polymorphic DNA (RAPD) method employs arbitrarily arranged short-engineered oligonucleotides (10 bp long) as primers to produce countless random DNA fragments using PCR. The enhanced DNA segments are separated with the use of agarose gel electrophoresis and visualized using ethidium bromide dye or SyBRgreen. The polymorphisms in the enhanced fragments are brought about by:

1) base replacement or excision at the preparation sites,[12]
2) primer insertions at loci too far apart to result in amplification or[13]
3) insertions or deletions that change the size of the amplified fragment.[14]

Due to its ease of use (no prior DNA sequence information is required), low expense, ability to produce a high volume of DNA markers within a brief time frame, and the use of non-complex equipment, RAPD has a wide scope of applications.[15]

Arbitrarily primed PCR

Arbitrarily primed PCR (AP-PCR) is a distinct form of RAPD that uses single primers of 10–50 bps in length during a 3-stage amplification process. The initial 2 annealing cycles are completed in a low-stringent environment, with the main cycle employing high-stringency conditions and primers of various length. Polyamide gel

Table 1. Comparison of DNA profiling techniques for herbal authentication and quality control.[1] The table is based on the most frequent molecular tools and does not represent all DNA-based molecular techniques[27]

Molecular technique	Importance of authenticity	Reproducibility	Quantity of DNA required	Level of polymorphism	Locus specificity	Technical demand	Sequence information required	Automation	Development cost	Running cost
ISSR	++	medium	low	medium	no	low	no	yes	low	low
AFLP	++	high	medium	medium	no	medium	no	yes	medium	low
ARMS	++	high	high	low	yes	low	yes	yes	low	medium
RAPD	+	low	low	medium	no	low	no	yes	low	low
RFLP	+	high	high	medium	yes	high	yes	no	high	medium
SSR	+	high	low	high	no	low	yes	yes	medium	high
RAMPO	+	medium	low	medium	yes	high	no	yes	medium	medium
CAPS	++	high	low	low	yes	low	yes	yes	low	medium
SCAR	++	high	low	low	yes	low	yes	yes	low	medium
CAPS	++	high	low	low	yes	low	no	difficult	medium	medium
LAMP	+	high	low	low	yes	medium	yes	yes	low	medium
SNP	+	high	low	high	yes	medium	yes	yes	low	high
Microarray	+++	high	low	high	yes	high	yes	yes	high	high
Barcoding	+++	high	low	low	yes	medium	yes	yes	medium	medium

ISSR – inter simple sequence repeat; AFLP – amplified fragment length polymorphism; ARMS – amplification-refractory mutation system; RAPD – random amplified polymorphic DNA; RFLP – restriction fragment length polymorphism; SSR – simple sequence repeats; RAMPO – random amplified microsatellite polymorphism; CAPS – cleaved amplified polymorphic sequences; SCAR – sequence-characterized amplified regions; LAMP – loop-mediated isothermal amplification; SNP – single nucleotide polymorphism.

electrophoresis is chiefly used to examine the obtained products. This method is utilized in a variety of genetic investigations and for species identification. However, similar to RAPDs, fingerprints produced using single primers can cause reproducibility issues since slight changes in cycling conditions can affect the appearance of the bands.[16]

DNA amplification fingerprinting

As an adaptation of the RAPD strategy, the technique of DNA amplification fingerprinting (DAF) in thermostable conditions allows primers to efficiently enhance amplification at multiple random sites on each DNA strand and to produce fingerprints with novel DNA patterns. Polyacrylamide gels and silver staining are used to resolve the amplicons.[17]

Inter simple sequence repeats

Inter simple sequence repeats (ISSRs), or single primer amplification reaction (SPAR) markers, are exceptionally adequate, replicable, profoundly polymorphic, and advantageous to use in higher plants. They are utilized in genetic fingerprinting, quality labeling, phylogenetic investigation, species and cultivator identification, and the evaluation of hybridization. The IISRs resemble RAPDs, though ISSR primers are located in microsatellite (SSR) regions and are longer in length (around 14 bp) than RAPD primers. The procedure uses basic sequence repeats (the ISSRs) with mono-, di- or trinucleotide repeats of 4–10 repeating units. The SSRs are abundant and highly polymorphic, and are distributed throughout the genome. Moreover, this method is efficient and more conservative. Also, the technique does not require sequencing data and does not use radioactive material.[18]

Amplified fragment length polymorphism

An amplified fragment length polymorphism (AFLP) is a powerful tool that combines RflM and PCR for DNA fingerprinting of the organismal genome and can screen countless loci (ca. 50–100 parts for each response) for polymorphisms. Similar to ISSR, AFLP does not require prior sequencing data for the targeted genome and is exceptionally reproducible. It is utilized for DNA fingerprinting, usually when very little data is available on the plant genome under study. Some advantages of AFLP include the rapid procedure, high genomic bounty, low labor requirements, abundant data, high reproducibility, and the ability to detect a high number of loci in a single reaction. Furthermore, no information about the target genome is required for preliminary development.[19]

Random amplified microsatellite polymorphism

Random amplified microsatellites (RAMS), or random amplified hybridization microsatellite (RAHM), is a mix of arbitrarily prepared PCR microsatellites (RAPDs) used for hybridization and production of polymorphic hereditary fingerprints, and does not require prior genomic information. As in RAPDs, the genomic DNA is initially amplified with a single random 10-mer primer or microsatellite-complimentary 15- to 16-mer PCR primer. This method produces highly reproducible banding patterns, which allow for clear identification of species-specific groups. Furthermore, random amplified microsatellite polymorphism (RAMPO) fragments are less sensitive to error than RAPD fragments due to their homology and the strong signal produced from the hybridization of distinct polymorphisms (i.e., the existence of repeat sequences in a particular microsatellite). The RAMPO is, for the most part, utilized for the identification and separation of genotypes within germplasms and populaces, such as *Ficus* and *Phoenix dactylifera*.[20]

Restriction fragment length polymorphism

Restriction fragment length polymorphism (RFLP) is widely utilized for recognizing changes at the DNA level and depends on the correlation of banding patterns from DNA sequences digested by specific restriction enzymes (e.g., HaeIII, EcoRI, BamHI). These enzymes are a class of endonucleases produced by microorganisms that can digest specific DNA sequences, with each protein recognizing a particular palindromic sequence. They detect and digest the DNA in a predictable manner, which generates restriction fragments of defined lengths. The length of these fragments varies between species if there are differences between the cleavage loci of a specific restriction endonuclease. Restriction sites are detected and then altered by point mutation, insertion, deletion, or translocation to produce distinct fragments that are separated and detected using gel electrophoresis. A few drawbacks of this method are that it is time-consuming, expensive, requires extensive labor, and needs large amounts of DNA.[21]

Microsatellites or single sequence repeats)

Microsatellites, short tandem repeats (STRs), or simple sequence length polymorphisms (SSLPs), are the smallest class of monotonous DNA sequences and have 2–6 bps. They are dispersed throughout most eukaryotic genomes, meaning there is a wide variety in the quantity of repeating units that are profoundly polymorphic. Microsatellites have a broad assortment of uses, such as marker-guided reproduction, population genetics and genome mapping. Indeed, the marker sequence and the extensive number of alleles allow for the detection of significant degrees of similarities and differences between closely related species or strains. A few downsides of microsatellites are that their use is time-consuming, and when the target DNA is unknown, there are high costs associated with the isolation and separation of each locus.[22]

Selectively amplified microsatellite polymorphic loci

The selectively amplified microsatellite polymorphic loci (SAMPL) strategy encompasses the high multiplexing ratio of the AFLP method and the high degree of microsatellite polymorphisms. The method uses AFLP-type primers matched to a complimentary microsatellite primer to detect polymorphisms using restriction digestion and selective amplification. The microsatellite primer restriction fragments that have linked adapter sequences are then amplified through primers complementary to the adapter sequences. A restriction of multi-locus SSR profiling is that it only detects a portion of the microsatellite polymorphisms due to the prevalence of predominant markers, which makes it difficult to distinguish allelic sections in complex DNA fingerprints.[23]

Directed amplification of minisatellite-region DNA

The directed amplification of minisatellite-region DNA (DAMD) strategy is essentially a DNA fingerprinting technique in which the loci of variable number tandem repeats (VNTR) of hypervariable repeats are enhanced at moderately high stringencies. The double repeat DNA sequences in minisatellites and VNTR are longer (around 10–60 bp themes) than microsatellites, though microsatellites display extensive polymorphisms due to their duplicate number of double repeat loci. The VNTR central sequence is utilized as a primer to coordinate the amplification of minisatellite loci DNA using PCR and can provide data similar to RAPD for species identification.[9] However, with the utilization of longer primers in DAMD, enhanced DNA fingerprinting is produced, and consequently, DAMD is more reproducible than RAPD.[24]

Single nucleotide polymorphisms

Single-base pairing locations in the genotypes of a minimum of 2 persons are termed single nucleotide polymorphisms (SNPs), at which various alleles are available. Single-base pair contrasts are created between DNA sequences through polymorphisms caused by point transformation. The SNPs are used for identifying people, ecotypes and species, and are proficient molecular markers for genetic examination and rearing projects. Environmental and transformative investigations also use SNPs. Furthermore, some molecular markers depend on SNPs, such as RFLPs, cleaved amplified polymorphic sequences (CAPS), amplification-refractory mutation system (ARMS), and single-strand conformation polymorphism (SSCP). Many great SNP sequences in exemplar DNA have been demonstrated in DNA microarrays (biotechnology organizations). Furthermore, SNPs are applied to the verification of *Perilla*, *Dendrobium officinale*, *Panax* cultivars, and *Boehmeria* species.[25]

Amplification-refractory mutation system

Amplification-refractory mutation system, or allele-specific polymerase chain response (AS-PCR), is a method for separating alleles. It is a simple, versatile and powerful technique for the detection of minor deletions or transformations, including SNPs. It uses a dynamic screening test that does not require any type of marking, because agarose gel electrophoresis and ethidium bromide staining are utilized for basic visualization of the amplified product. However, oligonucleotides with a random 30-mer sequence will not function as a PCR primer in the ARMS. Another disadvantage of the ARMS method is that it only amplifies the test DNA at the objective allele and does not enhance the non-target allele. In this procedure, the enhancement and approval steps are joined to such an extent that the presence or absence of the objective allele is analyzed by the presence or absence of a PCR product. Nonetheless, this procedure has been applied to the validation of *Alisma*, *Panax*, *Rheum*, *Dendrobium*, and *Curcuma*.[26]

Cleaved amplified polymorphic sequence

Cleaved amplified polymorphic sequence (CAPS) detects polymorphisms using a combination of target DNA amplification and restriction enzyme digestion in a 2-stage process. In the initial step, amplification of a characterized sequence uses specific 22–25-bp primers and is followed by restriction of the PCR product using suitable enzymes. Agarose gels and ethidium bromide are then used to isolate the separated restriction fragments. The capacity to distinguish DNA polymorphisms is higher for SSRs and AFLPs than for CAPS due to the limited size of the restriction fragments and the nucleotide changes that are fundamental for CAPS identification. For validation of *Astragalus*, *Alisma*, *Duboisia*, *Sinopodophyllum*, *Dysosma*, *Fritillaria*, *Ephedra*, *Artemisia*, *Panax*, *Actinidia*, *Atractylodes*, *Glehnia*, *Dendrobium*, and *Codonopsis*, PCR-RFLP has been used.[27]

Sequence-characterized amplified regions

Sequence-characterized amplified regions (SCAR) are another type of RAPD-determined molecular that avoids many of the shortcomings of RAPDs. Cloned polymorphic RAPD or ISSR fragments with specific oligonucleotide primers are utilized alongside SCAR markers to rapidly enhance nucleic acids indicative of natural materials. Polymorphic regions from the cloned RAPDs or ISSRs are selected from known sequences and used as primers to amplify and map SCAR markers. The SCAR recognize just a single locus, their amplification is less sensitive to reaction conditions, and they tend to exhibit dominance due to a partial overlap with the primers, so they are invaluable over RAPD markers. This strategy has been used for validation of *Panax* and separation of types of *Artemisia*,

Phyllanthus, *Pueraria*, *Sinocalycanthus*, *Embelia*, and *Lycium*.[28]

Single-strand conformational polymorphism

Single-strand conformational polymorphism is a transformation procedure. Under denaturing conditions, this method results in single-stranded DNA (ssDNA) folding into a tertiary conformation. The ssDNA is changed into the tertiary structure through the interaction of nucleotides in the DNA sequences, which also influence the mobility of the ssDNA during gel separation, with differences in bands indicating SNPs. The F-SSCP is a modified form of SSCP that includes theenhancement of the target sequence using fluorescent probes, and although this technique is not often applied for verification, it has been used for validating *Boesenbergia*.[29]

Methylation-sensitive amplified polymorphism

Methylation-sensitive amplified polymorphism (MSAP) is a modified AFLP method developed to screen genomic DNA methylation. The methylation-sensitive chemicals HpaII and MspI are used twice to digest genomic DNA, followed by the methylation-insensitive EcoRI. The digested samples are ligated using double-stranded DNA (dsDNA) adapters, pre-amplified using pre-selective or non-selective primers, and then amplified using a pair of selective primers. The MSAP was first developed to identify DNA methylation processes in dimorphic parasites and later adjusted for the recognition of cytosine methylation in the rice genome, pepper, apples, and Siberian ginseng.[7]

Loop-mediated isothermal amplification

Loop-mediated isothermal amplification (LAMP) is used to enhance target nucleic acids with high specificity and efficacy and is done under isothermal conditions. The method uses an auto-cycling process to segment and displace DNA, followed by hybridization carried out by a DNA enzyme with high helix displacement activity. It was applied to identify *Panax ginseng* and to distinguish the plant from *Panax japonicus* using *Ginseng radix*. It was subsequently utilized to pinpoint sites of *Lophophora williamsii* and *Curcuma longa*.[30]

Strand displacement amplification

Constrains of PCR-based methods for differentiating plants come from their low latency, though hybridization-based microarrays offer a rapid, high-throughput tool for genotype identification. In this regard, strand displacement amplification (SDA) is a technique created using combined genomics. The DNA library consists of 49 angiosperm plants in which all non-angiosperm genomic DNA was extracted using the Clontech® PCR-Select™ complementary DNA (cDNA) Deduction unit (Takara, Shiga, Japan). The SDA is superior to regular molecular identification techniques in terms of exactness, versatility and efficacy, as well as being high-throughput with expansive applications. Moreover, the technique exhibits substantial potential for genotyping, as demonstrated by the extraction of the non-angiosperm DNA. This review demonstrates the capability of setting up a high-throughput microarray-based autonomous genotyping procedure of significant angiosperm clades. The SDA procedure was suitable for 2 separate ginseng species, *P. ginseng* and *Panax quinquefolius*, which are often blended and contaminated. Furthermore, SDA was sufficiently sensitive to identify contamination debasement of 10% *P. quinquefolius* in *P. ginseng*.[31]

Multiplex ligation-dependent probe amplification

Multiplex ligation-dependent probe amplification (MLPA) is a semi-quantitative PCR-based method suitable for identifying clinical plants, and is a popular research tool because of its minimal expense and versatility. It utilizes the versatility of the PCR, though it broadens the specificity by including an important ligation step which ensures only DNA sequences that have been hybridized are detected. A typical PCR primer is used for the amplification of all target sequences, which is a critical element that ensures the general evaluation of each focus against a control sample.[32]

Ongoing polymerase chain reaction

Continuous quantitative PCR is a widely used method for measuring nucleic acids in molecular diagnostics. It offers advantages such as sensitivity, specificity and reproducibility. However, like any diagnostic method, it has its limitations. These limitations may include issues related to primer design, sample quality, amplification efficiency, and the potential for false-positive or false-negative results. It is important to carefully consider these limitations and optimize the experimental conditions in order to ensure accurate and reliable results when using continuous quantitative PCR in molecular diagnostics. It has become the principal specialized stage for nucleic acid discovery both in research and routine diagnostics. By estimating the buildup of amplified products during the PCR using fluorescent technology, it can identify a specific DNA sequence in a sample. The process has been used for the routine validation of the Chinese medicinal plant *Cimicifuga foetida* and its 4 substitutes: *C. simplex*, *C. dahurica*, *C. heracleifolia*, and *C. acerina*, through examination of recombinant DNA (rDNA) using an inner translated spacer (ITS) and fluorescence liquefying bend

investigation. In addition, continuous PCR has been used to investigate debasements of the genera *Euphorbia*, *Gentiana* and *Drynaria*.[33]

DNA sequencing analysis

The Sanger dideoxy-sequencing method was designed in the 1970s and is used for DNA sequencing. It is the most common way of generating reads of the bases or nucleotides, such as A, T, G, and C, of a specific molecule of DNA. The DNA is duplicated through chromosomal replication and can be carried out using capillary electrophoresis, in a cylinder, or on a microtiter plate with very small samples. The sequence read length is 50–1000 bps. If the loci to be sequenced outperforms the length of a standard sequencing read, internal reactions must be utilized to reproduce the total sequence of a more drawn-out DNA region.[34] Most DNA sequencing methods utilize slim electrophoresis to isolate DNA particles dependent on their size and fluorescent probes for labeling and identifying the 4 bases. Currently, Sanger dideoxy-sequencing is a widely used method in molecular biology with various applications. It is particularly valuable in studying phylogenetic relationships, population genetics, systematics, and advancing our understanding of genetic diversity. By sequencing DNA samples using the Sanger method, researchers can compare sequences and analyze genetic variations within and between populations. This information helps in elucidating evolutionary relationships, determining genetic markers, and studying the genetic basis of various traits or diseases. The versatility of Sanger dideoxy-sequencing allows researchers to explore a wide range of genetic questions and make significant contributions to the fields of phylogenetics, population genetics, systematics, and molecular biology as a whole. The sequencing examinations dependent on molecular ITS have been applied to *Panax*, *Yamaji*, *Fritillaria*, *Asarum*, *Astragalus*, *Dendrobium*, *Leonurus*, *Perilla*, *Phyllanthus*, *Rehmannia*, *Salvia*, *Swertia*, *Plantago*, *Bupleurum*, and *Euphorbia*. For validation of *Adenophora*, *Aconitum*, *Angelica*, *Astragalus*, *Curcuma*, *Epimedium*, *Fritillaria*, *Crocus*, *Ligularia*, *Pueraria*, and *Saussurea*, a 5S rDNA intergenic spacer marker has been utilized. Also, 18S rDNA has been evaluated in *Dioscorea*, *Pinellia* and *Panax* from atomic DNA.[3]

DNA barcoding

DNA barcoding is a novel molecular and bioinformatics tool with enormous scope. It is intended to provide fast, precise, automatable, and accurate species identification with high validity. This innovation requires a novel nucleotide sequence of small DNA fragments (400–800 bps), which are then used with specific reference sequences to distinguish samples and find unknown taxa. Also, it utilizes a shorter hereditary indicator from a regular locus (similar to a creature's plastidial DNA, atom or mitochondria). The ideal DNA marker should be useful for a wide scope of taxa (expansiveness of order application), readily retrievable with an all-inclusive preliminary duo, be sufficiently brief to be open to bidirectional sequencing, and provide exceptional grouping and maximal separation among species. The key example is the genus *Dendrobium* (Orchidaceae), which is represented by 78 varieties within Chinese botanical system, with 14 endemics. However, wide surface areas coupled with great morphological variability render species identification challenging.[16]

In light of preparation techniques, *Herba dendrobii* is characterized as a new *Dendrobium* as well as "Fengdou Shihu", along with "Huangcao Shihu", as it is the dominant type of *Herba dendrobii* in traditional Chinese medicine (TCM). A further noteworthy model where different varieties are utilized in TCM is the genus *Fritillaria* (Liliaceae), which incorporates 24 varieties and 15 species and is endemic in China. *Bulbus fritillariae* is the most famous homegrown prescription in China and is being utilized as an antitussive and expectorant herb.[35] The *Fritillaria* genus encompasses several species that are utilized in TCM, including *F. thunbergii*, *F. cirrhosa*, *F. unibracteata*, *F. przewalskii*, *F. delavayi*, *F. ussuriensis*, *F. walujewii*, *F. pallidiflora*, *F. hupehensis*, *F. anhuiensis*, and *F. puqiensis*. These species may have similar names, making it challenging for buyers to distinguish between them. Additionally, there may be a lack of specific and distinct attributes or characteristics associated with each species, further adding to the confusion. It is important to ensure accurate identification and quality control of these substances (the plant components) used in TCM to maintain their safety and efficacy. Therefore, for quality control and normalization of *Fritillaria* as a natural medication, DNA barcoding is the main reliable technique for the identification of accurate species.[36]

DNA microarrays (DNA chip technology)

The innovative "biochip" technology is intended to recognize fluorescently marked DNA or ribonucleic acid (RNA) fragments through their hybridization to oligonucleotides. The DNA microarray innovation offers a high pace of creation for the simultaneous assessment of numerous qualities in many taxa. This innovation is utilized for the identification and confirmation of natural medications, though we need to distinguish particular DNA sequences of interest in every species to design a standard test on a chip to use for comparisons. The DNA fragments of a normal sample are immobilized and organized on a microarray and attached with glass slides, silicon or nylon layers.[37]

Presently, DNA chip technology has been applied for the identification of different types of *Fritillaria*, *Dendrobium* and *Bupleurum*. Species-specific oligonucleotide assays were obtained through the 5S ribosomal RNA quality of *Euphorbia kansui*, *Pinellia ternata*, *Teucrium*

divaricatum, *Pinellia cordata*, *Aconiti kusnezoffii*, *Typhonium giganteum*, *Croton tiglium*, *Dysosma tatula*, *Dysosma pleiantha*, *Dysosma versipellis*, *Hyoscyamus niger*, *Pinellia pedatisecta*, *Datura inoxia*, *Rhododendron molle*, *Strychnos nux-vomica*, *Alocasia macrorrhiza*, *Datura metel*, and *Aconitum carmichaeli*.[38]

Examples of DNA-based validation of natural plants

Olive oil is sold with 20% or more fake oils, and as artificial and unsaturated fats are similar, it is difficult to separate them using standard methods. However, the complete genomic DNA was isolated using olive oil mixed with sunflower oil and canola, and was investigated for SNPs in non-coding loci of psbA-trnH and the incomplete coding area of the matK plastid. The amplification of these DNA loci was completed with specific primers using PCR, and the obtained DNA sequences were aligned to standardized tag sequences of the canola and sunflower oil DNA. This combination of molecular science methodology and bioinformatics technology is a practical strategy for guaranteeing the purity of olive oil by aligning contaminated DNA fragments with their standardized DNA after blending canola and sunflower oil into olive oil. Adulteration of up to 5% in olive oil can be quickly detected using this plastid-based sub-atomic DNA innovation.[39]

The genome sequence of *Parthenium argentatum* plastid was shown to have 152,803 bps. In addition, it was demonstrated that the genomic configuration of the *P. argentatum* chloroplast is similar to that of *Helianthus annuus*, based on the general connection of specific protein expression patterns. Unlike *Guizotia abyssinica*, *H. annuus* and *Lactuca sativa*, *P. argentatum* (guayule) is a latex-producing woody shrub and was marketed to individuals with type I latex hypersensitivity as a hypoallergenic protectant. The total chloroplast genomes of *L. sativa*, *H. annuus* and *G. abyssinica*, along with the plastid genome of *P. argentatum*, have more modest 3.4 kb reversals compared to the larger 23 kb matK and psbA-trnH spacer chloroplast DNA used for the standard identification of other *Parthenium* species: *P. schottii*, *P. argentatum*, *P. hysterophorus*, and *P. tomentosum*. When contrasted with the sequences of plastid genomes, namely *L. sativa*, *G. abyssinica*, *H. annuus*, and *Asteraceae*, the DNA-based validation method revealed distinction through the development of the 4 genomes. The improvement in chloroplast-specific DNA standardized probes was then used to identify *Parthenium* varieties.[40]

The RAPD has been widely used for the detection of genetic inconsistencies within plants. This approach was initially utilized to genetically identify 11 arid species of plants, including *Bassia eriophora*, *Caylusea hexagyna*, *Sonchus oleraceus*, *Zilla spinosa*, *Lycium shawii*, *Rumex vesicarius*, *Zygophyllum propinquum* ssp. *migahidii*, *Andrachne telephioides*, *Achillea fragrantissima*, *Withania somnifera*, and *Moricandia sinaica*, gathered in various areas of Saudi Arabia. Five primers were used to amplify the plant species DNA, and the RAPD profiles increased from 307 to 1772 bps. Pairwise comparisons between promotions, given the extent of shared homology created by the primers used, were determined with the assistance of the StatistiXL program v. 1.7 (https://www.statistixl.com/download/) using Jaccard's similitude coefficient. A significant value of 0.32 was shown between *L. shawii* and *A. fragrantissima*, and a least pairwise likeness of 0 was seen between *A. telephioides*, *Z. spinose*, *B. eriophora*, *B. eriophora*, and *Z. propinquum* when the 5 primers were consolidated.[41]

Boerhavia diffusa, also called Punarnava, is a herb native to India and is frequently used in traditional Indian medicine. Precise identification and clustering of *B. diffusa* are critical for improving the adequacy and biosafety of the medications prepared with the use of this herb. The DNA barcoding strategies are used to identify and isolate *B. diffusa* through closely related species. The phylogenetic examination of the 4 types of *Boerhavia* with potential for tagging included ribosomal DNA locales ITS, ITS2, ITS1, and the chloroplast plastid psbA-trnH. Grouping arrangement uncovered 26% polymorphic regions in ITS, 30% in ITS1, 16% in ITS2, and 6% in psbA-trnH, separately. A phylogenetic tree was built for 15 varieties using the ITS sequences that differentiated *B. diffusa* from different species. The ITS1 had a higher change/transversion proportion, level of variety and pairwise distance, which separate *B. diffusa* from different types of *Boerhavia*. The concentrate uncovered that the potential candidate regions that could be utilized for distinguishing *B. diffusa* and verifying its natural products were ITS and ITS1.[42]

Valerian (*Valeriana officinalis*) is a therapeutic herb generally utilized as a gentle sedative and anxiolytic. Among the vast number of synthetic constituents (such as flavonoids, alkaloids, terpenoids, and iridoids) found in valerian root, the active ingredients that are liable to have a soothing effect are valerenic acid (C15 sesquiterpenoid) and valerena-4,7(11)-diene. The NGS (Roche 454 pyrosequencing; Roche Diagnostics, Basel, Switzerland) was used to generate 1 million record reads of valerian roots to identify an active terpene synthase. Two sesquiterpene synthases were identified (VoTPS1 and VoTPS2) from the collected records, both of which demonstrated dominating articulation designs in the root. Transgenic yeasts VoTPS2 and VoTPS1 delivered germacrene C/germacrene D and valerena-4,7(11)-diene, separately, as significant terpene products. Purified VoTPS1 and VoTPS2 recombinant proteins affirmed these actions in vitro with pharmacokinetics (Km of ~10 μM and kcat of 0.01 s^{-1}) shown for the 2 chemicals. The design of the valerena-4,7(11)-diene created from the VoTPS2 signals was additionally validated through 13C-nuclear magnetic resonance (NMR) along with gas chromatography-mass spectrometry (GC-MS) in correlation with an engineered standard. Kumar et al.

described a methodology that includes cutting-edge sequencing and the use of metabolically designed organisms to understand terpenoid variety in restorative plants.[43]

Phyllanthus (Euphorbiaceae) varieties, well known due to their hepatic defensive action, are used in a few traditional medicines in native medical services in India. They are traded as crude homegrown medications. Samples of *Phyllanthus* that are used in crude drugs were acquired from 25 shops in south India and consisted of 6 unique varieties, such as *Phyllanthus amarus*, which were identified by examining their morpho-taxonomical properties and through molecular investigations. Testing revealed that 76% of the pharmaceutical assays consisted of *P. amarus* at a purity >95%, without admixtures. The remaining 24% of shops had 5 distinct species of *Phyllanthus*, including *P. maderaspatensis*, *P. fraternus*, *P. debilis*, *P. kozhikodianus*, and *P. urinaria*. The chloroplast DNA region, psbA-trnH, was used to generate species-specific DNA standardized tag marks for *Phyllanthus* species. The DNA scanner tag psbA-trnH locus of the chloroplast can successfully isolate *Phyllanthus* species and can be subsequently utilized to determine species admixtures in the raw drugs made from *Phyllanthus*.[44]

In this specific case, *Ruta graveolens*, which is sold as a dried restorative herb, was found to be contaminated with *Euphorbia dracunculoides*. The contamination of *Ruta graveolens* with *Euphorbia dracunculoides* represents an instance where the intended herb was adulterated or substituted with another species. This contamination highlights the importance of quality control and proper identification of herbs to ensure their safety and efficacy for consumers. The genomic DNA was extracted from leaves (100 mg each) using a modified cetyltrimethylammonium bromide (CTAB) protocol. The ITS sequences of ribosomal DNA (nrDNA-ITS) and chloroplast spacer sequences (rpoB and rpoC1) have favorable plant DNA barcoding qualities. These spacer groupings were amplified, sequenced and confirmed with a Basic Local Alignment Search Tool (BLAST) search. The sequence arrangements were made using ClustalX (http://www.clustal.org/clustal2/) to search for contrasts in the groupings. A DNA marker was then created dependent on rpoB and rpoC1 of the nrDNA-ITS to detect the *E. dracunculoides* contaminants in the *R. graveolens* samples. *Ruta graveolens* (289 and 264 bps) and *E. dracunculoides* (424 bps) sequences were created through different groupings of the nrDNA-ITS and amplified to accelerate the verification cycle. This marker effectively recognized the species in extricated assays using just 5 ng DNA/μL.[45]

Future developments

The development of new computerized methods and specific instruments for DNA examination, such as smaller-than-expected sequencing, nanoscale DNA sequencing, microsphere-based suspension cluster, and cutting-edge sequencing, will bring about further innovations. Other promising advancements include nanopore technology for the highly precise identification of DNA bases, and Zenith, an enzymatic genotyping tool for investigating tens to thousands of genomic variations in a single multiplexed response. Another emerging technique is the oligonucleotide ligation assay (OLA), which has a broad scope in the multiplex examination of nucleic acid sequences, and can discover known single SNPs and separate alleles in polymorphic genes. These novel ways to deal with DNA sequencing guarantee deep genomic investigation, exhibit a high multiplexing limit, and open up many new ways for genotyping and future taxon identification.[46]

The new high-throughput sequencing (HTS) advances allow for the development of new molecular methodologies. An equal sequencing innovation, delivering a large number of DNA sequences (altogether 0.5–60 giga base sets) in a solitary run, has transformed genomic research in science and medicine. These cutting-edge sequencing stages, such as the Roche 454, Illumina, Solexa, Helicos, and Applied Biosystems framework, can arrange DNA quicker and at much lower costs than the standard 96-well setup of Sanger sequencing.[47]

Cutting-edge sequencing strategies such as NGS can help answer new and long-standing questions. Even though no "third generation" methods have been made monetarily accessible yet, a few organizations have advanced models that propose dynamic concepts.[48]

Conclusions

The effort to identify specific types of medicinal plants and produce both conventional and novel natural compounds suitable for use in medical science requires accurate botanical, phytochemical and biochemical recognition methods.

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References

1. Wagner H, Ulrich-Merzenich G, eds. *Evidence and Rational Based Research on Chinese Drugs*. Vienna, Austria: Springer Vienna; 2013. doi:10.1007/978-3-7091-0442-2
2. Skalli S, Bencheikh RS. Safety monitoring of herb-drug interactions: A component of pharmacovigilance. *Drug Saf*. 2012;35(10):785–791. doi:10.1007/BF03261975
3. Li S, Han Q, Qiao C, Song J, Lung Cheng C, Xu H. Chemical markers for the quality control of herbal medicines: An overview. *Chin Med*. 2008;3(1):7. doi:10.1186/1749-8546-3-7
4. Altukhov Y, Salmenkova E. DNA polymorphism in population genetics [in Russian]. *Russian J Genet*. 2002;38(9):989–1008. doi:10.1023/A:1020288812170

5. Chawla H. *Introduction to Plant Biotechnology*. 3rd ed. Boca Raton, USA: CRC Press; 2011. doi:10.1201/9781315275369
6. Zhao Z, Hu Y, Liang Z, Yuen J, Jiang Z, Leung K. Authentication is fundamental for standardization of Chinese medicines. *Planta Med*. 2006;72(10):865–874. doi:10.1055/s-2006-947209
7. Lian CL, Wadud MdA, Geng Q, Shimatani K, Hogetsu T. An improved technique for isolating codominant compound microsatellite markers. *J Plant Res*. 2006;119(4):415–417. doi:10.1007/s10265-006-0274-2
8. Kumar P, Gupta V, Misra A, Modi D, Pandey B. Potential of molecular markers in plant biotechnology. *Plant Omics*. 2009;2(4):141–162. https://www.pomics.com/Pradeep_2_4_2009_141_162.pdf. Accessed February 15, 2023.
9. Silva LM, Montes De Oca H, Diniz CR, Fortes-Dias CL. Fingerprinting of cell lines by directed amplification of minisatellite-region DNA (DAMD). *Braz J Med Biol Res*. 2001;34(11):1405–1410. doi:10.1590/S0100-879X2001001100005
10. Heath DD, Lwama GK, Devlin RH. PCR primed with VNTR core sequences yields species specific patterns and hypervariable probes. *Nucl Acids Res*. 1993;21(24):5782–5785. doi:10.1093/nar/21.24.5782
11. Hamilton AC. Medicinal plants, conservation and livelihoods. *Biodivers Conserv*. 2004;13(8):1477–1517. doi:10.1023/B:BIOC.0000021333.23413.42
12. Luo YM, Zhang WM, Ding XY, et al. SNP marker and allele-specific diagnostic PCR for authenticating herbs of Perilla [in Chinese]. *Yao Xue Xue Bao*. 2006;41(9):840–845. PMID:17111830.
13. Ding G, Zhang D, Feng Z, Fan W, Ding X, Li X. SNP, ARMS and SSH authentication of medicinal *Dendrobium officinale* KIMURA et MIGO and application for identification of Fengdou drugs. *Biol Pharm Bull*. 2008;31(4):553–557. doi:10.1248/bpb.31.553
14. Warrier RR, Joshi G, Arunkumar AN. DNA fingerprinting in industrially important medicinal trees. *Ann Phytomed*. 2019;8(1):19–35. doi:10.21276/ap.2019.8.1.3
15. Wang G, Sun B, Zhu H, et al. Protective effects of emodin combined with danshensu on experimental severe acute pancreatitis. *Inflamm Res*. 2010;59(6):479–488. doi:10.1007/s00011-009-0152-1
16. Hollingsworth M. Book review: *DNA Fingerprinting in Plants: Principles, Methods and Applications*, 2nd ed. Weising K, Nybom H, Wolff K, Kahl G. 2005. Boca Raton: CRC Press. $99·95 (paperback). 472 pp. *Ann Botany*. 2006;97(3):476–477. doi:10.1093/aob/mcj057
17. Li C, Liu Z, Tian J, et al. Protective roles of Asperosaponin VI, a triterpene saponin isolated from *Dipsacus asper* Wall on acute myocardial infarction in rats. *Eur J Pharmacol*. 2010;627(1–3):235–241. doi:10.1016/j.ejphar.2009.11.004
18. Liang Z, He M, Fong W, Jiang Z, Zhao Z. A comparable, chemical and pharmacological analysis of the traditional Chinese medicinal herbs *Oldenlandia diffusa* and *O. corymbosa* and a new valuation of their biological potential. *Phytomedicine*. 2008;15(4):259–267. doi:10.1016/j.phymed.2008.01.003
19. Zhu S, Fushimi H, Cai S, Komatsu K. Species identification from ginseng drugs by Multiplex Amplification Refractory Mutation System (MARMS). *Planta Med*. 2004;70(2):189–192. doi:10.1055/s-2004-815502
20. Chatti K, Saddoud O, Salhi-Hannachi A, Mars M, Marrakchi M, Trifi M. Analysis of genetic diversity and relationships in a Tunisian fig (*Ficus carica*) germplasm collection by random amplified microsatellite polymorphisms. *J Integr Plant Biol*. 2007;49(3):386–391. doi:10.1111/j.1744-7909.2007.00396.x
21. Yang DY, Fushimi H, Cai SQ, Komatsu K. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and Amplification Refractory Mutation System (ARMS) analyses of medicinally used *Rheum* species and their application for identification of *Rhei rhizoma*. *Biol Pharm Bull*. 2004;27(5):661–669. doi:10.1248/bpb.27.661
22. Fugh-Berman A. Herb-drug interactions. *Lancet*. 2000;355(9198):134–138. doi:10.1016/S0140-6736(99)06457-0
23. Sasaki Y, Fushimi H, Cao H, Cai SQ, Komatsu K. Sequence analysis of Chinese and Japanese curcuma drugs on the *18S* rRNA gene and *trnK* gene and the application of amplification-refractory mutation system analysis for their authentication. *Biol Pharm Bull*. 2002;25(12):1593–1599. doi:10.1248/bpb.25.1593
24. Seyedimoradi H, Talebi R. Detecting DNA polymorphism and genetic diversity in Lentil (*Lens culinaris* Medik.) germplasm: Comparison of ISSR and DAMD marker. *Physiol Mol Biol Plants*. 2014;20(4):495–500. doi:10.1007/s12298-014-0253-3
25. Maeda M, Nishimura Y, Kumagai N, et al. Dysregulation of the immune system caused by silica and asbestos. *J Immunotoxicol*. 2010;7(4):268–278. doi:10.3109/1547691X.2010.512579
26. Semagn K, Bjørnstad Å, Ndjiondjop M. An overview of molecular marker methods for plants. *Afr J Biotechnol*. 2006;25(5):2540–2568. https://www.ajol.info/index.php/ajb/article/view/56080. Accessed February 15, 2023.
27. Heubl G. DNA-based authentication of TCM-plants: Current progress and future perspectives. In: Wagner H, Ulrich-Merzenich G, eds. *Evidence and Rational-Based Research on Chinese Drugs*. Vienna, Austria: Springer Vienna; 2012:27–85. doi:10.1007/978-3-7091-0442-2_2
28. Rhouma S, Dakhlaoui-Dkhil S, Ould Mohamed Salem A, et al. Genetic diversity and phylogenic relationships in date-palms (*Phoenix dactylifera* L.) as assessed by random amplified microsatellite polymorphism markers (RAMPOs). *Sci Hortic*. 2008;117(1):53–57. doi:10.1016/j.scienta.2008.03.013
29. Albani MC, Battey NH, Wilkinson MJ. The development of ISSR-derived SCAR markers around the seasonal flowering locus (SFL) in *Fragaria vesca*. *Theor Appl Genet*. 2004;109(3):571–579. doi:10.1007/s00122-004-1654-4
30. Orita M, Iwahana H, Kanazawa H, Hayashi K, Sekiya T. Detection of polymorphisms of human DNA by gel electrophoresis as single-strand conformation polymorphisms. *Proc Natl Acad Sci U S A*. 1989;86(8):2766–2770. doi:10.1073/pnas.86.8.2766
31. Munthali M, Ford-Lloyd BV, Newbury HJ. The random amplification of polymorphic DNA for fingerprinting plants. *Genome Res*. 1992;1(4):274–276. doi:10.1101/gr.1.4.274
32. Kurane J, Shinde V, Harsulkar A. Application of ISSR marker in pharmacognosy: Current update. *Phcog Rev*. 2009;3(6):216–228. https://www.phcogrev.com/article/2009/3/6. Accessed February 17, 2023.
33. Makino R, Yazyu H, Kishimoto Y, Sekiya T, Hayashi K. F-SSCP: Fluorescence-based polymerase chain reaction-single-strand conformation polymorphism (PCR-SSCP) analysis. *Genome Res*. 1992;2(1):10–13. doi:10.1101/gr.2.1.10
34. Techaprasan J, Klinbunga S, Jenjittikul T. Genetic relationships and species authentication of Boesenbergia (*Zingiberaceae*) in Thailand based on AFLP and SSCP analyses. *Biochem Syst Ecol*. 2008;36(5–6):408–416. doi:10.1016/j.bse.2007.10.006
35. Jayasinghe R, Kong S, Coram TE, et al. Construction and validation of a prototype microarray for efficient and high-throughput genotyping of angiosperms. *Plant Biotechnol J*. 2007;5(2):282–289. doi:10.1111/j.1467-7652.2007.00240.x
36 Holme IB, Dionisio G, Brinch-Pedersen H, et al. Cisgenic barley with improved phytase activity: Cisgenic barley. *Plant Biotechnol J*. 2012;10(2):237–247. doi:10.1111/j.1467-7652.2011.00660.x
37. Bartelson KJ, Singh RP, Foxman BM, Deng L. Catalytic asymmetric [4 + 2] additions with aliphatic nitroalkenes. *Chem Sci*. 2011;2(10):1940. doi:10.1039/c1sc00326g
38. Park HG, Yang Song J, Hwan Park K, Hwan Kim M. Fluorescence-based assay formats and signal amplification strategies for DNA microarray analysis. *Chem Eng Sci*. 2006;61(3):954–965. doi:10.1016/j.ces.2005.05.054
39. Murhadi WR. Good corporate governance and earning management practices: An Indonesian case. *SSRN Journal*. 2010. doi:10.2139/ssrn.1680186
40. Baldwin BG, Sanderson MJ, Porter JM, Wojciechowski MF, Campbell CS, Donoghue MJ. The ITS region of nuclear ribosomal DNA: A valuable source of evidence on angiosperm phylogeny. *Ann Missouri Bot Gard*. 1995;82(2):247. doi:10.2307/2399880
41. Jewell M. Clinical application of VASER-assisted lipoplasty: A pilot clinical study. *Aesthet Surg J*. 2002;22(2):131–146. doi:10.1067/maj.2002.123377
42. Selvaraj D, Shanmughanandhan D, Sarma RK, Joseph JC, Srinivasan RV, Ramalingam S. DNA barcode ITS effectively distinguishes the medicinal plant *Boerhavia diffusa* from its adulterants. *Genomics Proteomics Bioinformatics*. 2012;10(6):364–367. doi:10.1016/j.gpb.2012.03.002
43. Kumar S, Kahlon T, Chaudhary S. A rapid screening for adulterants in olive oil using DNA barcodes. *Food Chem*. 2011;127(3):1335–1341. doi:10.1016/j.foodchem.2011.01.094
44. Arif IA, Bakir MA, Khan HA, et al. Application of RAPD for molecular characterization of plant species of medicinal value from an arid environment. *Genet Mol Res*. 2010;9(4):2191–2198. doi:10.4238/vol9-4gmr848

45. Selvaraj D, Shanmughanandhan D, Sarma RK, Joseph JC, Srinivasan RV, Ramalingam S. DNA barcode ITS effectively distinguishes the medicinal plant *Boerhavia diffusa* from its adulterants. *Genomics Proteomics Bioinformatics*. 2012;10(6):364–367. doi:10.1016/j.gpb.2012.03.002
46. Pyle BW, Tran HT, Pickel B, et al. Enzymatic synthesis of valerena-4,7(11)-diene by a unique sesquiterpene synthase from the valerian plant (*Valeriana officinalis*). *FEBS J*. 2012;279(17):3136–3146. doi:10.1111/j.1742-4658.2012.08692.x
47. Srirama R, Senthilkumar U, Sreejayan N, et al. Assessing species admixtures in raw drug trade of *Phyllanthus*, a hepato-protective plant using molecular tools. *J Ethnopharmacol*. 2010;130(2):208–215. doi:10.1016/j.jep.2010.04.042
48. Al-Qurainy F, Khan S, Tarroum M, Al-Hemaid FM, Ali MA. Molecular authentication of the medicinal herb *Ruta graveolens* (*Rutaceae*) and an adulterant using nuclear and chloroplast DNA markers. *Genet Mol Res*. 2011;10(4):2806–2816. doi:10.4238/2011.November.10.3