**DNA Barcoding**

 **Abstract**

To identify species, DNA barcoding employs certain DNA sections. To better understand, preserve, and make use of the world's biodiversity, efforts are being made worldwide to create DNA barcodes for all categories of living things and make this information publicly available. The two coding regions that make up the genes rbcL and matK are the primary DNA barcode markers for terrestrial plants. Each plant that is DNA barcoded must have a herbarium voucher in addition to the rbcL and matK DNA sequences in order to produce high-quality databases. Users of the data should also have access to information on the DNA sequence quality, the utilized primers, and trace files. For each species, DNA barcoding should be performed on many individuals to ensure accuracy and account for intraspecific variation. In plants, plastid DNA (rbcL, matK, trnL, and trnH-psbA regions) and nuclear DNA (ITS and ITS2 regions) are often used in DNA barcoding.

### Keywords

### Plants, rbcl, matk, DNA Barcoding, Species Identification, PCR, DNA extraction

### Introduction

### Plant DNA barcoding has gained prominence as a recent scientific development and is frequently employed as a taxonomical or species identification technique. In the usage of medicinal plants, DNA barcoding is crucial for both product verification and identification purposes. It is a molecular technique aimed at species identification through standard DNA sequencing, has been a recurrent concern in the scientific community. In the context of land plants, a consensus has been lacking regarding the region(s) to be used for barcoding. To tackle this issue, a comprehensive evaluation of seven prominent candidates from the plastid DNA (atpF-atpH spacer, matK gene, rbcL gene, rpoB gene, rpoC1 gene, psbK-psbI spacer, and trnH-psbA spacer) has been conducted to provide a community recommendation for a standardized barcode.To this end, we have assessed their performance in regard to recoverability, sequence quality, and species discrimination. Our findings indicate that the 2-locus combination of rbcLmatK stands out as the optimal plant barcode. Thus, we propose this core 2-locus barcode as a universal framework for the routine use of DNA sequence data in identifying specimens and contributing to the discovery of previously overlooked land plant species.

**Significance and Identification of medicinal plant:**

The need for environmentally friendly solutions has recently increased across all trade sectors. As the worldwide society shifted increasingly toward complementary and alternative medicine, it also had an influence on the medical and healthcare sectors. Traditionally, plant identification has been based on clearly observable physical traits (Miller et al.,2016). Plants, like all other living things, are impacted by both their environment and their genetics. The environment affects a plant's metabolism, particularly secondary metabolism, which produces the majority of the therapeutic qualities (Briskin, 2000). The only major methods of plant identification prior to the development of contemporary molecular technologies were morphological and anatomical descriptions, which typically entails the description of variance for morphological attributes by professional taxonomist and trained technicians through experience. This common practice of identifying species is waning because of constraints including misinterpretation brought on by a lack of advanced knowledge, neglectance of morphologically cryptic taxa, and inefficiency brought on by insufficient morphological keys for certain life stages (Vohra and Khera, 2013).

**Importance of Medicinal Plants:**

The use of herbal medicine in healthcare has grown significantly during the last few decades. The global traffic, in unprocessed medicines has increased as a result. The market for herbal commodities is growing due to the global revival of traditional healthcare systems and the promotion of a healthy lifestyle. Medicinal plants are one of the fastest-growing subsectors of India's alternative medicine business. India is a huge hot point for biodiversity. Ayurveda and naturopathy are some of the time-tested, ancient medical systems of India that are still helpful to people today.

 About 80% of the world's population, according one of the WHO report, relies on plant or herbs -based medical systems for their basic healthcare requirements. 80% of the basic components required to make traditional medicines come from medicinal plants. Due to its health advantages and practicality, herbal medicines are a topic that is gaining more and more attention on a global scale. With a large diversity of medicinal plant species (about 45,500), India accounts for approximately 7%–8% of the world's biodiversity. Out of these, more than 8000 species from both upper and lower plant groups are thought to have medicinal value, and 960 species of medicinal plants are thought to be traded, with 178 species having annual consumption levels over 100 (Aneesh et al., 2009; Efferth and Greten, 2012; www.cbd.int)

 The correct usage and ongoing availability of authentic raw ingredients are key factors in the success of these medications. Many a times the chemical compounds present in plants vary within the same species because of some unavoidable external factors such as humidity, light, soil composition, temperature, and pH, storage conditions. If the samples are purposefully tampered with using a marker substance, this may be deceptive. The increased need for precise identification cannot be met by the current taxonomic procedures alone. The environment frequently affects how traits are expressed, making it challenging to quantify. The success of the herbal medication industry entirely depends upon quick and accurate scientific identification of the plants. At an industrial scale, identification of medicinal plant is a lengthy and time-consuming process.

**Challenges:**

Health risks might result from exposure to dangerous plants, thus accurate species identification is necessary to choose the best course of action. DNA barcoding can be utilized as a molecular method for species identification since consumed plants are too much degraded for visual inspection. The rbcL DNA marker was chosen for molecular identification in light of the universal primers, PCR and sequencing success rate, and variety of the dangerous plants. Using rbcL DNA barcodes, a reference DNA barcode library for 100 dangerous plant species was produced. At the genus and species levels, respectively, 100% and 89% species differentiation was seen for the dangerous plants contained in the library. Congeneric species made up all of the undifferentiated species.

**DNA Barcoding:**

Using a small, uniform section of the genome, DNA barcoding is a method for identifying species. DNA barcoding is a potent and reliable technique for quick species identification. Researchers may identify plant species, including those that may be novel to science, rapidly by focusing on precise DNA sequences from a defined section of the genome. The appropriate use of DNA barcoding facilitates the exploration, identification and documentation of plant diversity within biodiversity hotspots. This molecular-based technique allows for a comprehensive understanding of the distribution patterns of plant life in particular ecologically important areas. It strengthens our capacity to conserve and defend these priceless natural resources and adds to the corpus of knowledge on biodiversity hotspots (Lahaye et al. 2008). Standardization, simplicity, and scalability are the three main tenets of DNA barcoding. Rapid, time-saving, and automated species identification from a variety of herbal items is possible with DNA barcoding. When using this technique, the target gene's DNA sequence analysis is followed by the extraction of the sample's DNA, which contains high rates of nuclear substitutions that can distinguish between species that are closely related while remaining more or less the same for all individuals of the same species. Animal systems are where DNA barcoding was initially proposed and gained widespread acceptance. DNA barcoding includes sequencing a short fragment of the mitochondrial cytochrome c oxidase subunit I (COI) gene, known as "DNA barcodes," from specimens that are taxonomically unidentified and comparing the results with a library of DNA barcodes from organisms whose taxonomy is known. [DNA barcoding](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/dna-barcoding) is increasingly used to obtain taxonomic information about unidentified organisms.

On the other hand, DNA barcoding provides an alternative and workable taxonomic toolbox for quick and accurate identification. Now DNA is also utilized to create bioinventories and contribute in species identification. [1] The use of herbal medicine in healthcare has grown significantly during the last few decades. The global traffic in unprocessed medicines has increased as a result. The success of the herbal medication industry depends on quick and accurate scientific identification of the plant or plants. At an industrial scale, bulk identification is a lengthy and time-consuming operation. On the other hand, DNA barcoding provides an alternative and workable taxonomic toolbox for quick and accurate result. The two multinational efforts, Consortium for the Barcode of Life (CBOL) and the multinational Barcode of Life (iBOL), were striving to create DNA barcodes in plants. An extensive group of scientists called CBOL is actively searching the world's flora and wildlife for DNA barcodes. The iBOL is the biggest biodiversity genomics organization, and their goal is to establish DNA barcoding study as a discipline of international science. They kept up a Barcode of Life Data Systems (BOLD) reference library, a cloud-based data storage platform utilized for worldwide species identification. It has been suggested that the ideal barcode zone would have a high level of inter-specific rather than intra-specific species divergence ([Kress and Erickson, 2007](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9635000/#B74))

Currently, DNA barcoding stands as a highly effective and widely employed tool that facilitates the rapid and precise identification of various plant species. Orchidaceae, the second largest family of flowering plants, boasts a staggering diversity of over 700 genera and approximately 20,000 species, which are distributed across a vast majority of the globe. The accurate identification of orchids not only serves to promote the safe and optimal utilization of these plants, but it also constitutes an essential component of germplasm resource preservation and use. Within the context of this study, the DNA barcoding of four chloroplast genes (namely, matK, rbcL, ndhF, and ycf1) was undertaken, with the goal of furnishing a theoretical framework for the identification of orchid species, germplasm conservation, and innovative orchid utilization. Through an examination of the nucleotide replacement saturation of either single or combined sequences among the four genes, this study discerned that these sequences had reached a saturation state and were thus amenable to phylogenetic relationship analysis. As such, this study represents an invaluable resource for researchers, laying the foundation for the conservation, evaluation, innovative utilization, and protection of Orchidaceae germplasm resources.

The botanical and descriptive aspects of pharmacognosy were supplemented by medicinal and pharmaceutical chemistry for drug quality assurance testing. Phytochemical analysis methods, such as Fourier transform infrared spectroscopy, high-performance liquid chromatography, mass spectrometry, nuclear magnetic resonance spectroscopy and thin-layer chromatography, are generally used in the authentication of plant materials (Harborne, 1998; Meena Devi et al., 2010). For the past few years, CBOL has concentrated on finding a plant barcode that is universally informative. DNA barcoding has revolutionized taxonomy through the molecularization, computerization, and standardization of approaches (Casiraghi et al., 2010)

**Techniques** **used :**

A major concern in the creation and verification of herbal medicines has always been the quality control of a product. To overcome these traditional problems in the identification of the products, different molecular techniques have been developed. Since taxonomy and quality control of herbal medicines work hand in hand, several molecular approaches have been developed, improved, and are now dominating in the scientific community for taxonomic identification of plants. In the pharmaceutical sector, chemotaxonomy is frequently used in addition to taximetrics. Biochemical and molecular approaches for plant identification were created as a result of the inherent limits in the information that the morphological features could provide.

In recent times, the use of molecular markers (DNA- or protein-based) to identify the plants more precisely has captured the interest of the entire world (Buriani et al., 2012). The potent method of genetic fingerprinting is now generally accepted in many developed nations for quality control of multicomponent herbal medicines and their final products. Various methods have been utilized for genome-based medicinal plant authentication with potential uses. The majority of these methods were based on methods that identify nucleotide sequences that are unique to a particular species and determine the nucleotide sequence of one or more genetic loci in the plants of interest. Sequencing makes it possible to identify the genes that have the most recent common ancestor by comparing the relatedness of the gene sequences in the samples. Contrary to gel-based fingerprints, which need more sophisticated image analysis software, DNA sequence data may be stored as straightforward text strings in electronic databases like GenBank and simply mined using text-based bioinformatics techniques. The great advancement of massively parallel and clonal sequencing systems to extract sequence data from individual molecules within a complex source has brought a new level of species resolution and can be referred to as next-generation sequencing (NGS) (Mardis, 2013; Metzker, 2010). The cost of gel-based fingerprints was greatly reduced by sequencing-based technology and automated DNA sequencers. Sequences are the sole approach for taxonomic investigations that is suitable since they are a complete record of their own history, but molecular data has the same homology issues as morphological data (Karp et al., 1997). DNA microarray has gained popularity as a technique for verifying the authenticity of herbal medicine due to its high throughput, sensitivity, accuracy, specificity, and repeatability of transcriptomics applications (Chavan et al., 2006; Lo et al., 2012). Professionals now have access to a variety of innovative approaches for quick and accurate plant species identification because to recent developments in molecular genetics. Another reliable method was developed in large part as a result of the expanding advances in taxonomy and biotechnology. DNA barcoding is a brand-new technology that (Hebert et al. 2003a) suggested as a useful addition to the taxonomic toolkit. They promoted the use of DNA barcodes, or short DNA sequences from a specific area of the genome, for biological identification. It suggests using standard DNA locus sequencing as a method for identifying species. A standard DNA barcode should successfully provide high discriminating across species, be retrievable with a single primer pair, and be amenable to bidirectional sequencing. Many authors have proposed DNA barcoding as an integrated approach with classical taxonomy for species identification and authentication in the postgenomics era This invention raised a number of contentious issues regarding the nature and goals of systematics and its numerous subdisciplines (DeSalle, 2006; Lipscomb et al., 2003; Rubinoff et al., 2006). The universality and high resolution of DNA barcoding are due to the use of different DNA sections as markers. A marker must exhibit both high inter- and low intra-specific variability to have effective discriminating power. The 'DNA barcoding gap' refers to this variation in distance between intraspecific and interspecific groups.

The definition of strong DNA barcodes in plants has proven to be considerably more challenging than in mammals. The pair of chloroplast genes (rbcL + matK) was selected by the worldwide Consortium for the Barcoding of Life (CBOL) in 2009 as the suggested official barcode for plants. But this method has significant limitations. First, a distinct barcode gap and monophyletic species are prerequisites for any barcode system to be effective. The COI gene is employed in animals' mitochondria and chloroplasts, respectively it was also shown that, using chloroplast regions, maximum species discrimination would be around 70% and very variable among plant groups. Due to the fact that it shows more variants and enables the resolution of hybrid or closely related species, numerous scientists have more recently argued in favor of include the nuclear ITS region in this barcode. In order to assess the effectiveness of various barcode schemes, we examined the nuclear ITS region and various chloroplast genes (rbcL, matK, psaB, and psbC) in the genus Vanilla, a taxonomically complicated group. It was discovered that the official CBOL barcode system functioned only 76% effectively in Vanilla, and we show that ITS can be added to this barcode system to improve resolution (for closely related species and to the subspecies level) and detect hybrid species.

 For the success of DNA barcode, the barcode loci must have sufficient information to differentiate unambiguously between closely related plant species and discover new cryptic species. For herbal plant identification, matK, rbcL, trnH-psbA, ITS, trnL-F, 5S-rRNA and 18S-rRNA have been used as successful DNA barcodes. Emerging advances in DNA barcoding coupled with next-generation sequencing and high-resolution melting curve analysis have paved the way for successful species-level resolution recovered from finished herbal products. ***(Mishra et al., 2016).***

**Some molecular techniques and its potential function**:

1. DNA sequencing: It uses dideoxynucleotide triphosphates and thermostable DNA polymerase to create chain-termination sequences in a temperature-cycling configuration. (Kretz et al. 1994)
2. Arbitrarily primed (AP)-PCR and the direct amplification of length polymorphism (DALP):

 One or more genetic loci's nucleotide sequences are discovered and the sequences that are unique to a particular species are sequenced. Cao et al. (1996), Desmarais et al. (1998), Ha et al. (2001)

1. DNA microarray: In this technique the Species-specific oligonucleotide probes were designed from 5S ribosomal RNA gene sequence and immobilized on silicon chip. Target sequences were amplified and fluorescently labelled by asymmetric PCR (Carles et al. 2005), (Schena et al. 1998)
2. PCR–short tandem repeats (STR): The PCR-short tandem repeats (STR) approach is combined with the microchip electrophoresis method Qin et al. (2005)
3. Loop-mediated isothermal amplification (LAMP): Amplification carried out under isothermal reaction conditions using allele-specific primers designed, based on the 18S ribosomal RNA gene sequence (Sasaki et al. 2008)
4. Intersimple sequence repeat (ISSR) and simple sequence repeats (SSR): Utilizing PCR primers complementary to two nearby microsatellites, ISSR amplification is performed. SSR are created using a genetic library that has been enhanced with repetitive motifs. Following isolation and sequencing of microsatellite-containing clones, primer design and PCR amplification employing the chosen primer pair are carried out. (Sharma et al. 2008), (Su et al. 2008), (Tamhankar et al. 2009)
5. Quantitative real-time PCR (Q-PCR/qPCR: Real-time PCR is used with various analytic software to identify DNA by using a chosen genomic location. Xue et al. (2009), Xue and Xue (2008)
6. Random amplified polymorphic DNA (RAPD) and sequenced characterized amplified region marker (SCAR): Specific marker-defined polymorphic termini are sequenced, and primers are designed for specific amplification of a particular locus in targeted species. Cao et al. (2010), Ghosh et al. (2011), Gupta and Mandi (2013)
7. Restriction fragment length polymorphism (RFLP): A specific targeted gene's PCR-amplified product is exposed to restriction digestion using several restriction enzymes. The resulting DNA fragments are separated on an agarose gel electrophoresis, transferred to a membrane via a blotting method, and then detected using labelled probes on X-ray film. Biswas et al. (2013), Lin et al. (2012)
8. Amplification refractory mutation system (ARMS) and multiplex ARMS (MARMS): On the basis of the target species' sequencing data's discovered mutation site, allele-specific primer pairs were created, and MARMS was used to identify them. Chiang et al. (2012), Diao et al. (2009), Wang et al. (2011)
9. Specific expression subset analysis (SESA): SESA was performed using cDNA populations obtained from the target plant species' active component (therapeutically valuable tissue) and other important tissues, which serve as tester and driver, respectively. The collected ESTs were then put through computer analysis (Shukla et al. (2013)

There are some strategies used to overcome the difficulties of DNA barcoding in plants have been reviewed below to familiarize researchers with the current developments.

1. **Single-locus strategy**:

 The goal of (Wicke and Quandt's 2009) work was to create universal primers that would effectively amplify the trnK/matK region in terrestrial plants. DNA barcoding, which identifies plant species, depends on this area of the genome. For the primers to have a wide range of applications, the researchers created and tested them on a variety of plant species. The study is a useful resource for taxonomy and plant identification and matK distinguishes out among all the plastid areas employed in plant systematics because of its faster pace of change. Another study by Barthet and Hilu (2007) focused to work on the expression of the matK gene and its function. The essential gene for the proper functioning of the trnK/matK region in land plants is matK gene, which is widely used in DNA barcoding to identify plant species. The researchers investigated the expression patterns of the matK gene in many different plant species and tried to identify its role in different plant tissues. They also examined the evolutionary changes and adaptations that might have influenced the matK gene over time. The work offers important new insights into the molecular mechanisms underlying plant genetics and evolution by analyzing the functional and evolutionary properties of the matK gene. These discoveries help us understand plant biology more broadly and might have applications in the future for DNA barcoding and species identification. (Barthet and Hilu, 2007; Hilu et al., 2003; Wicke and Quandt, 2009). The study conducted by (Lahaye et al. 2008) analysed 1084 plant species (nearly 96% of orchid species) and it has shown a portion of the plastid matK gene could be a universal DNA barcode for flowering plants. Johnson and Soltis (1994) and Olmstead and Palmer (1994) study says that because of the almost equal distribution of substitution rates among the three codon positions, as opposed to most protein-coding genes where the rates are skewed towards the third codon position, matK's rate of substitution is three times higher at the nucleotide level and is six times higher at the amino acid level than that of rbcL. Despite numerous attempts, the lack of universal primer sets for all taxa and the challenges it presents during PCR have led some researchers to hypothesize that matK may not be functional in some taxa. This is especially true for nonangiosperms, where the lack of universal primer sets results in low PCR amplification success and a rapid rate of substitution. (CBOL, 2009; Hidalgo et al., 2004; Kugita et al., 2003)

1. **Multi-locus Barcode**

Because individual loci performed poorly in initial in silico and laboratory-based assessments, the most recent option that was successful was the use of a mix of barcodes. The CBOL's Plant Working Group evaluated the appropriateness of many leading potential markers before recommending matK and rbcL, a two-locus combination, as the primary plant barcode. (CBOL, 2009; Fazekas et al., 2008; Newmaster et al., 2008). Another combination particularly rbcL and trnH-psbA, the internal transcribed spacers of nuclear ribosomal DNA (nrITS/nrITS2) have also been evaluated for their potential (Ferri et al., 2008;). In the case of two loci, the conserved coding locus would fit well with the taxa of a community sample to create deep phylogenetic branches, it has been found. In contrast, the hypervariable area of the DNA barcode will align easily in the subclades of closely related species. In several situations, combining three loci did not increase discrimination above the best two-loci barcodes. Additionally, to save money and avoid the expenditures associated with combining three loci for big data sets, the two-loci barcode was declared the standard barcode for terrestrial plants.

DNA barcode technology is used for fast and accurate species identification in the forests across tropics and temperate zones. One of the study conducted, 183 plant species in National Nature Reserve of South China were sampled and sequenced, and the matK, rbcL, and psbA-trnH were employed to generate multi-locus barcodes. The psbA-trnH possessed the highest integral success rate, the product of sequencing recovery and correct species identification (75%), followed by matK (70%), and rbcL (56%). A combination of three-locus barcode (matK, rbcL and psbA-trnH) could identify greater than 87% of the total species, which is followed by two-locus barcode (85% for matK+psbA-trnH, 83% for rbcL+psbA-trnH, and 81% for matK+rbcL). A comparison was made with the previously published results from one subtropical forest plot (LFDP in Puerto Rico, 143 species) and two tropical forest plots (BCI in Panama, 296 species; and NRS in French Guiana, 254 species) to evaluate the universality and species identification correctness of the proposed DNA barcodes for these four forest plots. For the plots in tropics and subtropics, the sequencing success rate of rbcL, psbA-trnH and matK were 93% and 95.1%, 91.5% and 94.6%, and 68.5% and 79.7%, respectively. The combination of matK + rbcL showed a high identification capacity in geographically restricted regions in taxonomic groups, whereas the three-locus barcode had a high rate of correct species identification both in tropics (84%) and in subtropics (90%) (**Pei.N.C.2012).**

 **2)** **Next-generation biodiversity assessment using DNA metabarcoding**

 Species identification is a prerequisite for almost all empirical ecological investigations when collecting data. When many species are automatically identified from a single bulk sample of whole organisms or from a single environmental sample (soil, water, feces, etc.) including degraded DNA, this is referred to as DNA metabarcoding. It may be used with environmental samples from both the present and the past. The development of DNA metabarcoding has been aided by the accessibility of next-generation sequencing technologies and ecologists' demand for high-throughput taxon identification. DNA metabarcoding's potential power as it is now used is primarily constrained by its reliance on PCR and by the considerable expenditure required to create thorough taxonomic reference libraries. The current DNA amplification step will no longer be necessary, and thorough taxonomic reference libraries made up of repetitive ribosomal nuclear DNA and whole organellar genomes can be constructed using the carefully curated DNA extract collections kept up by standardized barcoding initiatives. (Taberlet et al ,2012)

# **3) Microfluidic Enrichment Barcoding (ME Barcoding)**

As we already know that there are several uses for DNA barcoding in classical taxonomy, ecology, forensics, food analysis, and environmental research to help species identification. We provide Microfluidic Enrichment Barcoding (MEBarcoding), a practical approach for high-throughput DNA barcoding in plants. The technique of Microfluidic Enrichment Barcoding is widely employed for effective high-throughput DNA barcoding in plants, and it is known for being both cost-effective and highly efficient.. This technique involves the utilization of state-of-the-art technologies such as the Fluidigm Access Array and Illumina MiSeq, which allow for the simultaneous amplification of DNA from multiple samples, thus generating extensive plant DNA barcodes and comprehensive databases. This method is a potent alternative to traditional PCR and Sanger sequencing, as it yields barcode libraries across diverse plant lineages. Moreover, this approach also entails the use of a microfluidic enrichment device that utilizes electroosmosis-induced pressure flow to enhance enrichment efficiency, enabling the detection of trace substances. This novel approach also facilitates the co-localization of DNA or RNA particles with known barcode oligonucleotides, thereby enabling the correlation of a cell's transcriptome or DNA with phenotype or drug effects. Overall, Microfluidic Enrichment Barcoding represents a state-of-the-art technique that has transformed the field of high-throughput DNA barcoding in plants and holds significant promise in various biological applications. During a single thermal cycling cycle, MEBarcoding employs the Fluidigm Access Array to concurrently amplify specified areas for 48 DNA samples and hundreds of PCR primer pairs (generating up to 23,040 PCR products). We created a microfluidic PCR process utilizing the Fluidigm Access Array and Illumina MiSeq as a proof of concept. For each of the four main DNA barcode loci in plants—rbcL, matK, trnH-psbA, and ITS—we examined 96 samples. Building a reference library for 78 families and 96 genera from all main plant lineages—many of which are currently absent from public databases—was accomplished using this method. Results demonstrate that this method is a productive substitute for conventional PCR and Sanger sequencing to produce huge numbers of plant DNA barcodes and construct more thorough barcode databases. (Gostel et al.,2020)

**Some recommended DNA barcode loci for families of medicinal plants:**

1. rbcLa + matK + trnH-psbA : Combretaceae (Gere et al. (2013)
2. ITS/ITS2 + psbA-trnH, ITS and ITS2: Apiaceae (Liu et al. (2014)
3. atpF-atp + psbK-psbI + trnH-psbA: Orchidaceae (Kim et al. (2014)
4. ITS2: Araliaceaes (Liu et al. (2012b)
5. ITS2; matK; rbcL: Zingiberaceae (Shi et al. (2011), Vinitha et al. (2014)
6. ITS2: Rutaceae (Luo et al. (2010)
7. trnH-psbA: Polygonaceae (Song et al. (2009)
8. ITS2: Rosaceae (Pang et al. (2011)
9. trnH-psbA: Polygonaceae (Song et al. (2009)
10. ITS: Nyssaceae (Wang et al. (2012)
11. atpF-atpH: Lemnaceae (Wang et al. (2010)
12. trnL and ITS2: Leguminaceae (Madesis et al. (2012)
13. psbA-trnH: Lauraceae (Liu et al. (2012c)
14. matK and trnH-psbA: Lamiaceae (Theodoridis et al. (2012)
15. ITS2: Fabaceae (Gao et al. (2010a)
16. matK: Juglandaceae (Xiang et al. (2011)
17. ITS2: Asteraceae (Gao et al. (2010b)
18. matK + rbcL + trnH-psbA: Arecaceae (Yang et al. (2012)
19. rbcL + matK: Angiosperms (CBOL (2009)

**Conclusion:**

To replace the traditional identifying methods, DNA barcoding-based adulteration detection is still being developed. In contrast to DNA marker-based identification, which is better for validating the original species, chemical studies are used to find foreign substances and to check the quality of herbal medicines. To fully comprehend adulteration in herbal medications, DNA barcoding-based authentication must be combined with metabolomics, transcriptomics, and proteomics technologies. In order to include the DNA barcoding process in the standards for certifying herbal goods, this sector needs a strong scientific community. To test DNA barcoding-based herbal pharmacovigilance, close cooperation between national pharmacopeia authorities and university or commercial institutes specialists in DNA barcoding should be promoted. Along with chemical analytical techniques, regular DNA barcoding authentication might improve the quality and authenticity of the herbal business and make pharmacovigilance monitoring and signal detection easier. All herbal businesses will eventually adopt DNA barcoding-based authentication, with biomonitoring employing readily accessible barcodes to identify adulterants. As biological data is developing quickly, many DNA barcoding difficulties will also be overcome.

In the practical application of DNA barcoding in poisoning cases, it may be deemed appropriate to utilize genus-level identification. Through our rigorous analysis, we have derived that rbcL can serve as a primary marker in such cases. Furthermore, should the need arise, ITS2 or trnH-psbA may be employed as a secondary marker to facilitate identification of the poisonous plants. This comprehensive investigation serves as a fundamental basis for the development of a dependable molecular approach that can be utilized to accurately discern the poisonous species from the vomit samples of poisoning cases.

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Biochemical profile and biological properties of some Tunisian medicinal plants

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