DNA Barcoding in Medicinal Plant Authentication: Principles, Applications and Future Perspectives

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ABSTRACT

Plant medicinal provides therapy or medicine for the prevention and treatment of diseases. Many medicinal plants are used as source of therapy in modern medicine. However, correct identification of many medicinal plants is often difficult due to apparent morphology, extensive habitats and increasing adulteration in herbal products. Traditional taxonomy-based identification method is found sometimes unfruitful, especially when plant material is processed or chopped. Molecular approaches, especially DNA barcodes using small portions of plant DNA, have been proposed as efficient and reliable techniques for identification and authenticity assessment of medicinal plant species. This review discusses the principles and applications of DNA barcoding technology for the identification and authentication of medicinal plants. We mainly focus on three commonly used barcode regions: rbcL and matK from chloroplast DNA, and the nuclear internal transcribed spacer (ITS). We describe the workflow of DNA extraction, PCR amplification and sequencing, and sequence analysis using bioinformatics tools. Case studies demonstrate the role of the DNA barcodes in detecting adulterants, in distinguishing closely related species and in quality control in the herbal medicine industry.

Despite its numerous advantages, the method has some limitations including dependence on reference databases, high cost of sequencing and some technical challenges associated with the method. However, promising outlooks for the future based on integration of next-generation sequencing technologies and metabarcoding approaches are discussed. DNA barcoding provides an accurate, fast and reproducible means for the identification of medicinal plants. In addition to its relevance to pharmaceutical safety, the method can provide a powerful tool for biodiversity conservation and standardization of the quality of commercial herbal medicinal products.

Keywords: DNA barcoding; Medicinal plants; rbcL; matK; ITS; Plant authentication; Phylogenetic analysis

INTRODUCTION

Medicinal plants have long been sources of traditional medicine and continue to be an important area of research for modern drug discovery and development. Despite the availability of conventional medicines, a large proportion of the global population continues to use plant-based products as primary healthcare options, particularly in developing countries. The therapeutic properties of these plants are dependent on correct identification and proper quality assurance. However, with the increasing demand, globalization of herbal trade and large-scale commercialization, many problems such as adulteration, misidentification and substitution have emerged, and these practices are known to compromise safety and efficacy (World Health Organization, 2013). Despite continued consumption and growing demand for their medicinal value, the taxonomic complexity of medicinal plants has become a considerable obstacle to their proper identification and quality assurance. To address these difficulties, the use of DNA barcoding has been proposed as a promising approach for plant authentication. DNA barcoding enables species identification with high accuracy, even from very small quantities of highly processed material, thus facilitating the detection of adulterants. Furthermore, it can contribute to the documentation and conservation of biodiversity and supports both current and future

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regulations, by providing a scientifically validated tool for the authentication of herbal medicines on the global market (Seberg & Petersen, 2009).

DNA barcoding offers several advantages for the identification of plant species, including rapid processing of samples, high degree of automation and the production of reproducible results. However, there are also limitations to the method such as the quality of available reference databases, the lack of a universally acceptable barcode for all plant species and some experimental restrictions. However, as the methods for DNA sequencing and bioinformatics analysis are continually improving the method becomes more reliable. Recent advances in DNA metabarcoding and next-generation sequencing technologies have also dramatically expanded the applicability of DNA-based identification methods to complex herbal mixtures and environmental samples (Taberlet et al., 2018). Identification of medicinal plants based on macroscopic and microscopic characteristics, and by means of phytochemical tests, is a common approach in phytoanalytical science. However, the accuracy of such tests is often restricted by the unavailability of fresh specimens or processed material, such as teas, pills, and tinctures, that have undergone chemical, thermal, or mechanical degradation, which require differential approaches for their analysis. Although numerous plant species are currently being used as medicines, generally, the application of molecular identification methods is increasing, due to their reliability, reproducibility, and objectivity, as observed by (de Boer et al., 2015).

Identification of species is merely the tip of the iceberg. The application of DNA barcoding is diverse and ranges from the pharmacognosy of medicinal plants to the conservation of endangered species and compliance of regulatory requirements. The increased complexity in traded herbal products, and the rapid growth of the international market necessitates monitoring of their authenticity. Traditional morphological methods are insufficient to authenticate these products and hence a combined approach of DNA barcoding and NEXT GENERATION SEQUENCING along with a robust bioinformatics platform is urgently required. This will lead towards an efficient, high-throughput approach for the identification and medicinal plant authentication (Mishra et al., 2016).

Importance of Medicinal Plants

Medicinal plants have long been used as the primary source of therapeutic agents in both conventional and modern medical practices. Today, a significant portion of the world's population depends on medicinal plants as a source of medicines for the treatment of various diseases. Medicinal plants contain a wide variety of bioactive compounds such as alkaloids, flavonoids, terpenoids, and phenolic compounds, which are the basis of their pharmacological activity (Pharmacognosy). However, the increasing trend in the use of herbal medicines has resulted in the development of challenges in their authenticity and quality (Kumar et al., 2022).

Medicinal plants contain a wide range of bioactive compounds, including phenolic compounds, tannins, alkaloids, saponins, cardiac glycosides, steroids, terpenoids, flavonoids, phlobatannins, anthraquinones, and reducing sugars, which contribute to their therapeutic properties. However, the type of phytochemicals may vary in different medicinal plants. Phytochemicals in medicinal plants are involved in the therapeutic value of the plants in the management of various conditions such as mastitis, boils, hemorrhoids, congestion, headache, hepatitis, liver problems, vertigo, stomatitis, kidney problems, vision problems, and various conditions such as anemia and coughs in humans and animals (Agidew, 2022). These phytochemicals are produced in medicinal plants through a variety of metabolic pathways (Al-Khayri et al., 2023). Apart from their pharmacological importance, medicinal plants are essential in the maintenance of physical, mental, and spiritual health in different cultures. They include plants that have clinically proven medicinal compounds that may be used directly or as a precursor for the development of medicine, as well as those that are traditionally believed to have medicinal value (Davis and Choisy, 2024). Furthermore, medicinal plants are significant in that they have been used in the preservation of cultural traditions, maintaining indigenous knowledge systems that have been used for generations in the management of different health conditions (Asiminicesei et al., 2024).

Phytochemicals are generally classified into primary and secondary metabolites. Primary metabolites include essential compounds such as sugars, amino acids, chlorophyll, and nucleic acids, which are vital for the plant’s growth and development. On the other hand, secondary metabolites include bioactive phytochemicals such as alkaloids, flavonoids, terpenes, phenolics, lignans, steroids, curcumins, saponins, and glycosides. Of all the

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phytochemicals, phenolic compounds are the most dominant and account for about 45%, while terpenoids and steroids, alkaloids, and other compounds account for 27%, 18%, and 10%, respectively. Phytochemicals have substantial nutraceutical value and can be regarded as bioactive agents that can bridge the gap between the food and pharmaceutical industries (Nwozo et al.,2023). In addition to their medicinal value, the plants are also utilized as food, beverages, and cosmetics. Conventionally, botanists have employed various approaches in the identification of plants. However, the approaches are often challenging and require a lot of effort and accuracy. Plant classification is often based on the identification of various parts of the plants such as the roots, flowers, and leaves. Although leaves can be employed in the identification of plants owing to their unique shapes, texture, and color, the leaves of various species of plants may appear similar and may change in various stages of development (Azadnia et al., 2022).

Currently, the demand for medicinal plants has exerted pressure on natural populations, resulting in over-exploitation and stress. These conditions have led to a reduction in genetic diversity in species, which is a threat to sustainability and conservation (Duta-Cornescu et al., 2023). Despite these challenges, medicinal plants are readily available in the market and are considered to have fewer side effects compared to synthetic drugs. These conditions have elevated the importance of medicinal plants globally (Laldingliani et al., 2022).

Challenges in Medicinal Plant Authentication

To ensure the safety, efficacy, and quality of medicinal plants, their identification is of the utmost importance. However, the identification of medicinal plants using traditional methods such as the study of morphology and anatomy is not reliable, especially in the case of processed forms of medicinal plants such as powder, extract, and tablets. Moreover, the risk of misidentification is higher in the case of medicinal plants that are closely related and possess similar morphological characteristics. In the herbal drug market, adulteration and substitution are major issues that can result in decreased therapeutic activity or adverse effects (Sharma et al., 2021). Another complex issue is standardization of botanical extracts, and this is usually hampered by adulterated or improperly identified botanical materials. This, in turn, affects the concentration of active constituents of herbal medicines, hence affecting their efficacy. As identification of botanicals is mainly done by observing their characteristics, it is not only a tedious task but is also prone to human errors. As traditional medicine mainly relies on plant extracts, evidence-based methods are required for validating this system and for developing new therapeutic agents (Nabi et al., 2026).

Furthermore, the complexity of the authentication process is increased by the variability of medicinal plants arising from factors such as geographical location, seasonality, environment, and the diversity of the species. This variability makes it hard to develop standardized authentication processes (Urumarudappa et al., 2025). Moreover, the diverse sources of herbal products, which are often based on local and traditional usage, pose problems in defining and regulating herbal products, thus the inconsistencies in the quality of the products and the proliferation of dietary supplements and herbal medicine in the market (Travadi et al., 2024).

Currently, various quality evaluation and authentication techniques are mainly based on morphological characterization and phytochemical investigation, as described in various pharmacopoeias. Although such techniques are helpful in identifying specific marker compounds, they are sometimes not sufficient for tracing the biological origin of highly processed herbal preparations that might contain several constituents. Molecular techniques have also been explored as alternative tools for herbal authentication. Nevertheless, such techniques also have limitations. One of the limitations is that the lack of comprehensive and curated reference databases might restrict the accuracy of species identification (Raclariu et al., 2018). Incomplete and incorrect information in public databases can lead to incorrect identifications, which might compromise accuracy. In addition, genetic variations within species and similarities between closely related species can sometimes make species discrimination difficult, especially in large and complex plant families with minimal genetic divergence (Hollingsworth et al., 2011).

These technical issues also affect the efficacy of molecular authentication. Contamination in sample collection, DNA extraction, or PCR can affect the quality of results, which may result in false identification (Kress et al., 2005). All these issues highlight the need to develop standard protocols with stringent quality control in any molecular study. Moreover, limited access to sophisticated molecular techniques and high-throughput

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sequencing technologies in developing countries may hinder the application of these techniques in DNA-based plant authentication, creating a gap between research developments and implementation (Li et al., 2020). The lack of universally accepted barcode regions in all plant species also poses a threat to the efficacy of these techniques, as different barcode regions may be required for different plant species to ensure accurate identification (CBOL Plant Working Group et al., 2009). Closely related or recently diverged species are the most difficult to distinguish, especially in plants, where the selection of appropriate barcode regions and universality of primers are critical issues in plant identification (von Cräutlein et al., 2011).

Dna Barcoding as A Molecular Tool

DNA barcoding is a molecular-based technique that allows for species identification through the amplification and analysis of a standard region of DNA, which is often derived from genic or intergenic regions of extranuclear genomes. Although these regions are a small fraction of the total cellular DNA, regions of the chloroplast and mitochondrial genomes used for plant and animal barcoding, respectively, have sufficient nucleotide diversity to allow for the identification of the taxonomic identity of most species of interest to agriculture and medicine (Barcaccia et al., 2016). In recent years, DNA barcoding has become a widely accepted and efficient means of species identification through the use of a standard region of DNA. In plants, these regions include genes found in the chloroplast genome, such as rbcL and matK, and nuclear regions like ITS, which have a good balance of interspecific divergence and intraspecific conservation (Hebert et al., 2003).

One of the greatest advantages of DNA barcoding is that it can help to circumvent the limitations of traditional taxonomy because it can help in identifying species even when only a little is available. DNA barcoding can also help in identifying sterile specimens, young plants, poor-quality samples, and plant samples that are available only in the form of powders, tablets, and herbal formulations. Moreover, DNA barcoding can also help in sharing taxonomic information across the globe. Although barcode libraries have been developed for several important medicinal plant families and biodiverse regions, a large percentage of medicinal plant species is underrepresented. Moreover, the barcode regions that are currently being used are not entirely universal and may not be able to distinguish between closely related species. This is a critical disadvantage because there are several plant species that are of similar medicinal importance. (Davis and Choisy, 2024).

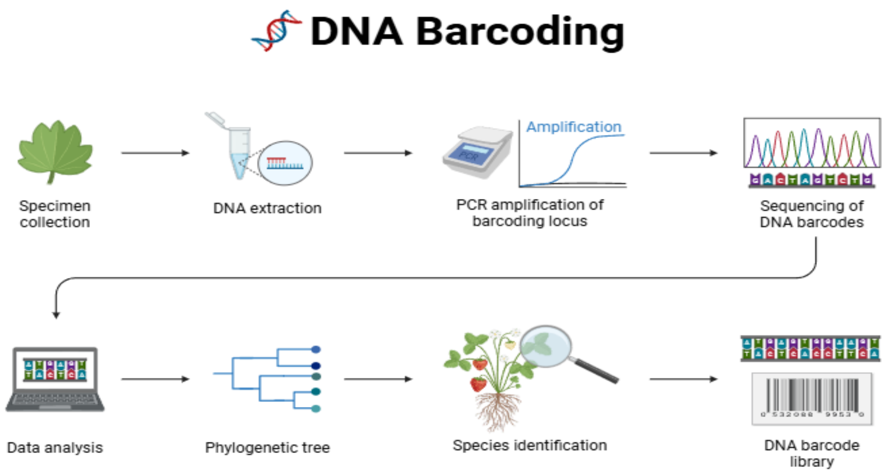

Fig 1. General workflow of DNA barcoding showing DNA extraction, PCR amplification, Sequencing and Sequence analysis (Created with Biorender.com).

The use of standardized barcode markers such as COI in animals and rbcL, matK, and ITS in plants, along with the development of comprehensive reference libraries, has greatly improved species identification. With the development in sequencing technologies and the use of bioinformatics tools, it is now possible to obtain barcode data quickly and effectively (Antil et al., 2023). In addition to this, the barcode regions can now be obtained even from minute amounts of DNA, which further adds to the efficiency of the technique. Apart from species identification, DNA barcoding has also contributed to the discovery of new species, as well as the understanding of the evolutionary relationships between species (Abdi et al., 2024). The development of global databases such as the Barcode of Life Data Systems (BOLD) has significantly facilitated the accessibility, standardization, and sharing of data, thereby facilitating the identification of species in different parts of the world (Ratnasingham & Hebert, 2007). More recently, the development of portable sequencing technology has facilitated the identification of species in the field without the need for sophisticated laboratory infrastructure (Pomerantz et al., 2018).

Apart from the identification of species, DNA barcoding has also played a vital role in pharmacovigilance by ensuring the safety of herbal medicine through the identification of the species used in the formulation of the drug (Newmaster et al., 2013). The development of mini-barcode technology has also facilitated the identification of degraded samples where the full-length barcodes cannot be obtained (Meusnier et al., 2008). Furthermore, the identification of species has also facilitated the identification of the pattern of genetic variation and the distribution of species in different regions of the world (Hajibabaei et al., 2007). The development of a combination of chemotaxonomic and metabolomic approaches has also facilitated the identification of plants in the field of herbal medicine (Ichim et al., 2020).

Applications in Medicinal Plant Authentication

The use of DNA barcoding in medicinal plant authentication has also witnessed much attention in recent times due to its reliability and accuracy. Many researchers have demonstrated its potential in the detection of adulterants, differentiation of closely related species, and quality control in medicinal plants. The technique has been largely used to authenticate herbal medicines, thus improving the quality and safety of medicinal plant-based drugs (Raclariu et al., 2018). Apart from its contribution to medicinal plant authentication, DNA barcoding plays a vital role in the conservation of biodiversity through accurate species identification and the maintenance of medicinal plant resources. It can be regarded as a potent technique with potential applications in taxonomy, systematics, ecology, conservation biology, and other fields (Abdi et al., 2024).

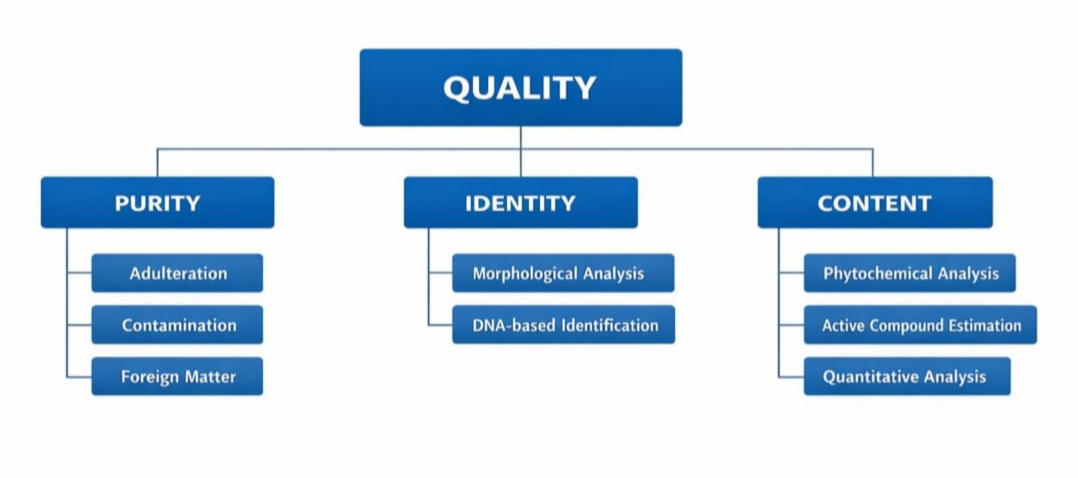

Fig 2. Flowchart representing the framework for quality evaluation of medicinal plants.

In the pharmaceutical field, DNA barcoding is being increasingly used to authenticate raw materials at the early stages of drug development, ensuring that plant-based materials are genuine and that there is little chance of contamination (Newmaster et al., 2013). It is also commonly used for quality assurance in herbal supplements by validating labels and ensuring that a particular plant species is present in a product (Coghlan et al., 2012). Moreover, this technique has been found to be extremely effective in authenticating Ayurvedic and other herbal products, wherein plant components are accurately identified in a formulation, ensuring that traditional herbal products are more reliable and effective in modern healthcare systems (Seethapathy et al., 2015). DNA barcoding is also extremely useful in ensuring consumer safety by detecting any toxic or allergenic plant species that may be present unintentionally in herbal products (Galimberti et al., 2013). It helps to clarify any taxonomic confusion that may be present in species complexes wherein identification is not possible using physical characteristics (Chen et al., 2010). It is also useful in quality control of plant materials wherein physical characteristics change over a period of time (Saslis-Lagoudakis et al., 2015).

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In addition to quality control, DNA barcoding also plays a role in the preservation of indigenous knowledge by scientifically proving the plant species used in ethnobotany activities (de Boer et al., 2014). It also plays a role in agriculture by helping to identify medicinal plant cultivars, thus improving the efficiency of cultivation (Parveen et al., 2016). The technique has been used in traditional Chinese medicine to authenticate ingredients in very complex mixtures, thus improving safety and standardization (Liu et al., 2018).

Moreover, the use of DNA barcoding is essential in the ecological and regulatory applications. In this regard, it helps in the identification of exotic plant species from medicinal plants, thereby avoiding any imbalance in the ecosystem. In addition, it ensures the sustainable use of plant resources (Gaskin et al., 2011). In the quality certification process, the use of DNA barcoding helps in the identification of pharmaceutical-grade herbal medicines using authentic DNA profiles (Sgamma et al., 2017). Moreover, the use of DNA barcoding helps in the breeding process by ensuring the purity of elite cultivars in the mass cultivation process (Sucher & Carles, 2008). In addition, it plays a significant role in the identification of microorganisms associated with plant materials, thereby enhancing the quality of herbal medicines (Ivanova et al., 2016).

Scope of the Present Review

This review seeks to provide an in-depth and updated account of the importance of DNA barcoding in the authentication of medicinal plants, with special emphasis on the safety, efficacy, and quality of herbal products. It provides a detailed account of the basic principles of DNA barcoding, with special emphasis on the application of standardized DNA sequences and the increased application of molecular tools in the identification of species. It provides special emphasis on the application of the rbcL, matK, and nuclear ITS sequences in the identification of plant species and their limitations in the identification of different species of plants.

This review also provides a detailed account of the process of DNA barcoding in the authentication of medicinal plants, with special emphasis on the major steps involved in DNA barcoding, such as DNA sequencing and analysis using databases such as GenBank and BOLD. In addition, the review provides an account of the various applications of DNA barcoding in the authentication of medicinal plants, such as the identification of adulteration and the application of DNA barcoding in biodiversity conservation.

Moreover, the advantages of using DNA barcoding over other traditional methods of taxonomy are presented, such as the accuracy and reliability of the DNA barcoding process in handling processed materials. However, the problems associated with the application of DNA barcoding in taxonomy are also presented in the review. These problems include the reliability of the databases, the lack of universally recognized DNA barcodes, and the technical problems associated with the process. Finally, the recent developments in the field of DNA barcoding and the future prospects of using the process in the identification of medicinal plants with the help of other omics approaches are presented.

CONCLUSION

DNA barcoding has the potential to identify adulteration, substitution, and contamination in herbal preparations, which has significantly improved the quality control process. In addition, the application of DNA barcoding in the pharmacognosy and standardization process of herbal drugs has improved the regulatory process by providing accurate verification of raw materials as well as the final product. Although DNA barcoding is associated with numerous advantages, there are also a few limitations. These limitations include a lack of universally applicable barcode regions for all plant species, limited availability of reference DNA sequences, and difficulties in DNA recovery from processed herbal products. In addition to this, cost, standardization, and access may also pose limitations. In conclusion, DNA barcoding is a highly effective and continually improving technique in medicinal plant authentication that is vital in quality assurance, safety evaluation, and regulatory compliance. With further advances in technology and its incorporation with other alternative analytical techniques, DNA barcoding is anticipated to increasingly contribute to the authentication, safety, and sustainability of medicinal plant resources in the coming years.

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