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DNA BARCODING AND MOLECULAR PHARMACOGNOSY: EMERGING TOOLS FOR AUTHENTICATION OF MEDICINAL PLANTS

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Abstract:

Medicinal plants have long been recognized as an important source of therapeutic agents and continue to play a significant role in traditional and modern healthcare systems. A large proportion of the global population relies on herbal medicines for the prevention and treatment of various diseases. However, the growing demand for herbal products has raised serious concerns regarding the authenticity, quality, and safety of medicinal plant materials. Problems such as misidentification, adulteration, and substitution frequently occur during the collection, processing, and commercialization of herbal drugs, which may reduce therapeutic efficacy and pose potential health risks. Conventional pharmacognostic methods including morphological, microscopic, and phytochemical evaluations are commonly used for the identification of medicinal plants, but these approaches often show limitations, particularly when plant materials are processed or closely related species exhibit similar characteristics.

In recent years, molecular approaches have emerged as reliable tools for the authentication of medicinal plants. Among these, DNA Barcoding has gained considerable attention as a rapid and accurate method for species identification using standardized DNA sequences. DNA barcoding utilizes specific genetic markers such as rbcL, matK, ITS, and trnH-psbA to differentiate plant species based on their unique genetic signatures. These molecular techniques enable the identification of plant materials even in powdered or processed forms and facilitate the detection of adulterants in herbal products.

This review highlights the importance of molecular pharmacognosy and DNA barcoding in medicinal plant authentication, discusses commonly used genetic markers, and outlines recent technological advancements and applications in herbal drug quality control. Integration of molecular tools with conventional pharmacognostic methods can significantly improve the accuracy, safety, and standardization of herbal medicines.

Keywords: *DNA barcoding; Molecular pharmacognosy; Medicinal plant authentication; Herbal drug quality control; Genetic markers; rbcL; matK; ITS; Adulteration detection; Herbal medicines.*

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INTRODUCTION:

Medicinal plants have been utilized as therapeutic agents for thousands of years and continue to play a crucial role in healthcare systems across the world. Traditional medical practices such as Ayurveda, Traditional Chinese Medicine, and Unani Medicine have long relied on plant-derived materials for the prevention and treatment of various diseases. These natural resources contain diverse bioactive compounds including alkaloids, flavonoids, glycosides, terpenoids, and phenolic constituents that contribute to their pharmacological activities. Even in modern medicine, numerous important drugs such as anticancer agents, analgesics, and antimalarial compounds originate from plants or their derivatives. The increasing interest in natural products and plant-based therapies has further strengthened the relevance of medicinal plants in contemporary pharmaceutical research and drug discovery.

Global Reliance on Herbal Medicines

A substantial portion of the global population depends on herbal medicines for primary healthcare. According to reports from the World Health Organization, nearly 70–80% of people in developing countries rely on plant-based remedies for their healthcare needs. The popularity of herbal medicines is attributed to their perceived safety, cultural acceptance, affordability, and availability. Additionally, the global herbal product market has expanded significantly in recent decades, driven by growing consumer preference for natural and holistic therapeutic approaches. Herbal formulations are widely used for the management of chronic diseases, immune support, and general wellness. However, the increasing demand for medicinal plants has also intensified concerns regarding quality, safety, and authenticity of herbal materials used in pharmaceutical preparations.

Problems Associated with Misidentification, Adulteration, and Substitution of Medicinal Plants

One of the major challenges in the herbal drug industry is the accurate identification of medicinal plant species. Misidentification may occur due to morphological similarities among closely related species or due to lack of proper taxonomic knowledge during collection and processing. Furthermore, intentional or unintentional adulteration and substitution are common issues in herbal raw materials. Adulteration refers to the inclusion of inferior, unrelated, or contaminated plant materials, whereas substitution involves replacing the authentic medicinal plant with another species that may possess similar physical characteristics but different chemical properties. Such practices may compromise the therapeutic efficacy of herbal formulations and may also pose potential safety risks to consumers. Therefore, reliable authentication techniques are essential to ensure the purity and quality of medicinal plant materials.

Limitations of Traditional Pharmacognostic Methods

Conventional pharmacognostic approaches used for plant identification primarily include macroscopic evaluation, microscopic analysis, and phytochemical screening. While these methods are useful for the preliminary authentication of crude drugs, they possess several limitations. Morphological characteristics may vary depending on environmental conditions, geographical origin, or developmental stages of the plant. Similarly, microscopic analysis requires specialized expertise and may become ineffective when plant materials are processed into powders or extracts. Phytochemical profiling can provide information about the presence of chemical constituents, but it does not always allow precise identification of plant species because many compounds are shared among different plants. Consequently, these traditional techniques alone may not provide sufficient accuracy for the authentication of medicinal plants, particularly in complex herbal products.

Emergence of Molecular Pharmacognosy and DNA Barcoding as Reliable Tools

Advancements in molecular biology have introduced new strategies for the accurate identification of medicinal plants. Molecular pharmacognosy integrates genetic and molecular techniques with traditional pharmacognostic approaches to improve the authentication of plant materials. One of the most promising techniques is DNA Barcoding, which involves the use of standardized DNA sequences as molecular markers to identify plant species. DNA barcoding relies on specific genomic regions that exhibit sufficient variation between species while remaining conserved within the same species. This method provides highly reliable identification even when plant materials are processed, fragmented, or morphologically indistinguishable. The availability of genetic databases such as GenBank and Barcode of Life Data System has further enhanced the application of DNA barcoding in medicinal plant authentication and biodiversity studies.

The primary aim of this review is to provide a comprehensive overview of the application of DNA barcoding and molecular pharmacognosy in the authentication of medicinal plants. The review discusses the significance of accurate plant identification in the herbal drug industry and highlights the limitations associated with conventional pharmacognostic methods. It also

explores the principles, methodologies, and molecular markers used in DNA barcoding for plant identification. In addition, the review summarizes recent advancements, applications, and challenges associated with molecular authentication techniques. By compiling current knowledge in this field, the article aims to emphasize the importance of integrating molecular tools with traditional pharmacognostic approaches to ensure the quality, safety, and efficacy of medicinal plant-based products.

Table 1: Key Issues and Modern Solutions in Medicinal Plant Authentication

Aspect	Description	Limitations/Problems	Modern Solution
Medicinal plant usage	Plants used as therapeutic agents in traditional and modern medicine	Increasing demand leads to overharvesting and misidentification	Scientific authentication and cultivation strategies
Global reliance on herbal medicines	Large population depends on plant-based medicines for healthcare	Quality control and safety concerns	Standardization and regulatory guidelines
Misidentification	Incorrect identification due to similar morphology	Reduced therapeutic efficacy	DNA-based species identification
Adulteration	Addition of inferior or unrelated plant materials	Safety risks and reduced drug quality	Molecular authentication methods
Substitution	Replacement of genuine plant species with other species	Loss of pharmacological activity	Genetic marker analysis
Traditional pharmacognostic methods	Identification based on morphology, microscopy, and phytochemistry	Not reliable for processed materials	Integration with molecular pharmacognosy
Molecular pharmacognosy	Application of molecular biology in pharmacognosy	Requires specialized equipment	Provides precise genetic identification
DNA barcoding	Species identification using short DNA sequences	Database limitations in some plants	Reliable authentication of medicinal plant species

CONCEPT OF PHARMACOGNOSY AND MOLECULAR PHARMACOGNOSY

Pharmacognosy is a specialized branch of pharmaceutical sciences that deals with the study of crude drugs obtained from natural sources such as plants, animals, and microorganisms. Traditionally, pharmacognosy focuses on the identification, classification, cultivation, collection, processing, and evaluation of medicinal plants and their derived products. It encompasses the study of morphological characteristics, microscopic features, chemical constituents, and pharmacological activities of natural drugs. Historically, pharmacognosy played a crucial role in the development of many therapeutic agents derived from plant sources. In herbal medicine and natural product research, pharmacognosy provides essential knowledge for the authentication, standardization, and quality assessment of medicinal plant materials used in pharmaceutical preparations.

Transition from Classical Pharmacognosy to Molecular Pharmacognosy

Classical pharmacognosy mainly relied on morphological examination, organoleptic evaluation, microscopic studies, and phytochemical analysis to identify medicinal plants. While these techniques remain valuable, they often present limitations when plant materials are processed into powders, extracts, or formulations where diagnostic features are lost. With the advancement of biotechnology and molecular biology, pharmacognosy has evolved into a more sophisticated discipline known as molecular pharmacognosy. This modern approach integrates genetic and molecular tools to study medicinal plants at the DNA level. Molecular pharmacognosy enables precise species identification, assessment of genetic diversity, and detection of adulteration in herbal materials. Techniques such as polymerase chain reaction (PCR), DNA sequencing, and molecular marker analysis have significantly improved the accuracy and reliability of plant authentication.

Role of Molecular Biology in Plant Identification

Molecular biology has revolutionized the identification and classification of medicinal plants by providing methods that analyze genetic information directly. Unlike morphological traits, which may vary due to environmental influences or developmental stages, DNA sequences remain relatively stable within a species. Molecular identification techniques use specific regions of the plant genome to differentiate species based on genetic variation. One of the most widely adopted approaches is DNA Barcoding, which employs short, standardized DNA fragments as molecular markers for species recognition. The use of

molecular markers allows researchers to identify plant species even in powdered or processed forms, where conventional identification methods are ineffective. Additionally, molecular techniques facilitate phylogenetic studies, genetic conservation programs, and the authentication of medicinal plant materials used in pharmaceutical industries.

Importance of Molecular Techniques in Quality Control of Herbal Drugs

Quality control is a critical aspect of herbal drug development and manufacturing. The therapeutic efficacy and safety of herbal medicines depend largely on the authenticity and purity of the plant materials used. Molecular techniques provide reliable tools for ensuring the quality of herbal products by enabling precise identification of plant species and detection of adulterants. DNA-based methods are highly sensitive and can detect even small quantities of contaminant species in herbal formulations. Furthermore, molecular authentication methods support regulatory requirements and standardization processes recommended by organizations such as the World Health Organization, which emphasizes the importance of proper identification and quality control in herbal medicines. By integrating molecular techniques with traditional pharmacognostic methods, researchers and pharmaceutical industries can improve the consistency, safety, and effectiveness of herbal drug products.

MEDICINAL PLANT AUTHENTICATION: NEED AND CHALLENGES

Issues in Herbal Drug Authentication

Authentication of medicinal plants is a fundamental step in ensuring the quality and safety of herbal medicines. Accurate identification of plant species is essential because different species may possess varying chemical compositions and pharmacological activities. However, several challenges exist in the authentication process, including inadequate taxonomic knowledge, variations in plant morphology, and improper handling during harvesting and processing. In many cases, raw herbal materials are traded in dried or powdered forms, making visual identification difficult. These factors contribute to errors in identification and compromise the quality of herbal drug preparations. Therefore, reliable authentication methods are necessary to maintain the integrity of herbal medicines.

Adulteration and Substitution in Herbal Medicine

Adulteration and substitution are among the most common problems encountered in the herbal drug market. Adulteration refers to the addition of inferior, unrelated, or contaminated materials to herbal drugs, either intentionally for economic gain or unintentionally during collection and processing. Substitution, on the other hand, involves replacing a genuine medicinal plant with another species that resembles it in appearance but may differ chemically and pharmacologically. These practices reduce the therapeutic value of herbal medicines and may also lead to adverse health effects. The presence of adulterants in herbal products undermines consumer trust and poses significant challenges for regulatory authorities responsible for ensuring product quality and safety.

Morphologically Similar Species

Many medicinal plants exhibit close morphological resemblance to other species within the same genus or family, making their identification difficult through visual examination alone. For example, closely related species may share similar leaf shapes, flower structures, or root characteristics, which can lead to confusion during collection and processing. In such cases, traditional identification methods based solely on external features may not provide sufficient accuracy. Molecular techniques offer a reliable solution by enabling species identification based on genetic characteristics rather than physical appearance.

Influence of Environmental Factors on Plant Morphology

Environmental conditions such as climate, soil composition, altitude, and seasonal variations can significantly influence the morphological characteristics of plants. These environmental factors may alter the size, color, and shape of plant organs, leading to variations within the same species. As a result, plants collected from different geographical regions may exhibit noticeable differences in appearance, complicating their identification through traditional pharmacognostic methods. Such variations highlight the need for more precise and stable identification techniques that are independent of environmental influences.

Impact on Efficacy, Safety, and Regulatory Compliance

Incorrect identification or adulteration of medicinal plants can have serious consequences for the efficacy and safety of herbal medicines. When the wrong species is used, the expected therapeutic effect may be reduced or completely absent. In some cases, substituted or adulterated materials may contain toxic compounds that can cause harmful side effects. Moreover, improper authentication of herbal drugs may lead to non-compliance with regulatory standards established by organizations such as the World Health Organization and the Indian Pharmacopoeia Commission. Therefore, accurate authentication

methods are essential to ensure product safety, therapeutic reliability, and regulatory approval.

Examples of Commonly Adulterated Medicinal Plants

Several medicinal plants commonly used in traditional medicine are known to be susceptible to adulteration or substitution. For instance, Panax ginseng is sometimes substituted with other species of ginseng or related plants with lower medicinal value. Similarly, Curcuma longa may be adulterated with other Curcuma species or artificial coloring agents to enhance appearance. Another example includes Withania somnifera, which is occasionally substituted with morphologically similar plant materials. These examples highlight the importance of reliable identification methods, including molecular techniques, to ensure the authenticity and quality of medicinal plant products used in healthcare and pharmaceutical industries.

Table 2: Comparison of Classical and Molecular Approaches in Medicinal Plant Authentication

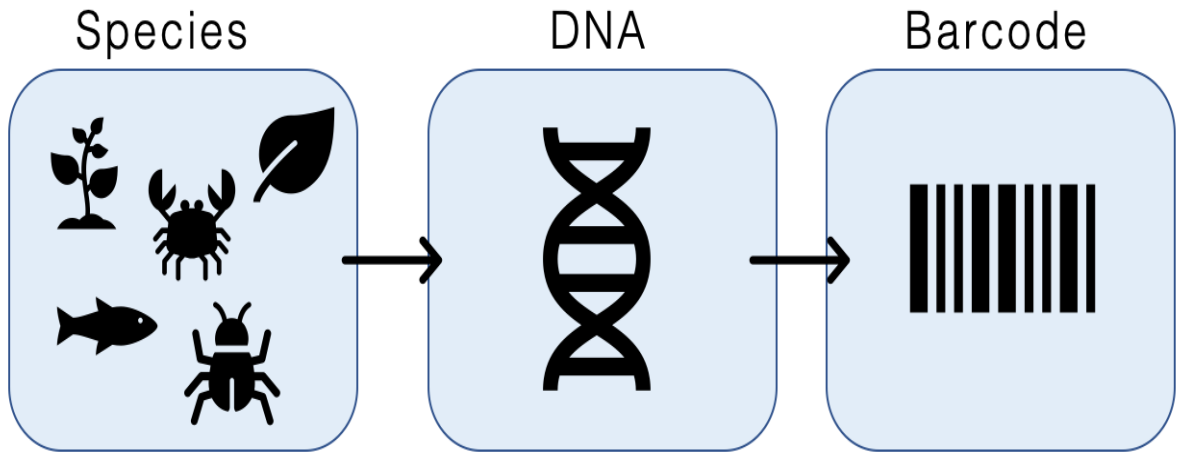
Parameter	Classical Pharmacognosy	Molecular Pharmacognosy
Basis of identification	Morphological, microscopic, and phytochemical characteristics	DNA sequence and genetic markers
Reliability	Moderate; affected by environmental conditions	High accuracy and species-specific
Identification of processed materials	Difficult or sometimes impossible	Possible even in powders or extracts
Expertise required	Taxonomic and microscopic expertise	Molecular biology and genetic analysis
Detection of adulteration	Limited capability	Highly sensitive detection
Influence of environment	Strong influence on morphology	DNA remains relatively stable
Application in industry	Preliminary authentication	Advanced quality control and regulatory compliance

DNA BARCODING: PRINCIPLES, WORKFLOW AND GENETIC MARKERS USED IN MEDICINAL PLANT AUTHENTICATION

Principle of DNA Barcoding

DNA barcoding is a modern molecular technique used for the identification and classification of biological species using short and standardized DNA sequences. The concept is based on the principle that each species possesses unique genetic variations in specific regions of its genome, which can serve as a molecular signature for identification. By analyzing these short DNA fragments, researchers can accurately determine the species origin of biological samples.

The concept of DNA Barcoding was proposed as a universal approach to species identification and biodiversity studies. Unlike traditional taxonomic methods that rely on morphological characteristics, DNA barcoding utilizes genetic information that remains relatively stable across environmental conditions and developmental stages. This method is particularly useful for identifying plant materials that are processed, fragmented, or morphologically indistinguishable.



DNA barcoding involves sequencing a small, standardized region of DNA and comparing it with reference sequences stored in global genetic databases such as GenBank and Barcode of Life Data System. If the sequence obtained from the unknown sample matches a reference sequence in the database, the species can be accurately identified. This molecular approach has become an important tool for authenticating medicinal plants, detecting adulterants, and supporting biodiversity conservation.

Workflow of DNA Barcoding for Medicinal Plant Identification

The process of DNA barcoding in medicinal plant authentication follows a systematic workflow that involves several molecular and bioinformatic steps. Initially, plant samples are collected and preserved properly to maintain DNA integrity. Genomic DNA is then extracted from plant tissues such as leaves, roots, or dried herbal materials.

After DNA extraction, specific barcode regions of the genome are amplified using polymerase chain reaction (PCR) with region-specific primers. The amplified DNA fragments are then sequenced using modern sequencing technologies. Once the DNA sequence is obtained, it undergoes editing and alignment to remove errors and ensure accuracy. The final sequence is compared with reference sequences available in global databases to determine the species identity.

This workflow enables researchers to identify plant species with high precision, even when the plant material is present in powdered or processed form. Consequently, DNA barcoding has become an important tool for quality control and authentication in the herbal drug industry.

Genetic Markers Used in DNA Barcoding of Medicinal Plants

In plant DNA barcoding, specific regions of the genome known as barcode markers are used for species identification. These markers are selected based on characteristics such as universality, ease of amplification, and sufficient variability between species. The most commonly used barcode regions in plants are located in chloroplast DNA and nuclear DNA.

Chloroplast DNA Markers

Chloroplast DNA regions are widely used in plant barcoding because they are relatively conserved and easy to amplify across different plant taxa.

rbcL gene:

The rbcL gene encodes the large subunit of the enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO), which is involved in photosynthesis. This gene is highly conserved and can be amplified easily across many plant species. Although rbcL provides good universality, its species-level discrimination ability is sometimes limited due to lower genetic variability.

matK gene:

The matK gene is located in the chloroplast genome and plays a role in RNA splicing during chloroplast gene expression. Compared with rbcL, matK exhibits higher evolutionary rates and therefore provides better species discrimination. However, amplification of matK may sometimes be difficult in certain plant groups due to primer limitations.

trnH-psbA intergenic spacer:

This chloroplast region is highly variable and is often used as a supplementary barcode marker for plant identification. The trnH-psbA spacer provides greater discriminatory power for closely related species and is frequently combined with other markers for improved accuracy.

trnL intron:

The trnL region is another chloroplast marker commonly used in plant identification studies. It is particularly useful in degraded samples because shorter fragments of this region can be amplified, making it suitable for analyzing processed herbal products.

NUCLEAR DNA MARKERS

Internal Transcribed Spacer (ITS):

The Internal Transcribed Spacer region is located within nuclear ribosomal DNA and consists of ITS1, ITS2, and the 5.8S rRNA gene. The ITS region shows higher variability than many chloroplast markers, making it highly useful for distinguishing closely related plant species.

ITS2 region:

The ITS2 subregion has gained popularity as a universal barcode marker for medicinal plants because of its short length, high variability, and reliable amplification across different taxa. It has been widely applied in the authentication of herbal medicines and detection of adulterants.

Importance of Multiple Marker Approach

In many cases, a single barcode marker may not provide sufficient discriminatory power to distinguish closely related plant species. Therefore, researchers often employ a combination of markers such as rbcL and matK or ITS with chloroplast markers. The use of multiple genetic markers increases identification accuracy and provides stronger evidence for species authentication.

This multilocus barcoding approach has significantly improved the reliability of medicinal plant identification and has become a standard practice in molecular pharmacognosy research.

Table 3: Common DNA Barcode Markers Used in Medicinal Plant Identification

Marker	Genome Location	Type of Region	Advantages	Limitations
rbcL	Chloroplast genome	Protein coding gene	High universality and easy amplification	Limited species discrimination
matK	Chloroplast genome	Protein coding gene	High evolutionary rate and better species resolution	Amplification difficulty in some plants
trnH-psbA	Chloroplast genome	Intergenic spacer	High variability, useful for closely related species	Sequence alignment difficulties
trnL	Chloroplast genome	Intron region	Works well for degraded DNA samples	Lower discrimination power
ITS	Nuclear genome	Ribosomal DNA spacer	High variability and species discrimination	Possible fungal contamination
ITS2	Nuclear genome	Subregion of ITS	Short length and high amplification success	Requires careful sequence analysis

APPLICATIONS OF DNA BARCODING IN AUTHENTICATION OF MEDICINAL PLANTS

Identification of Raw Medicinal Plant Materials

DNA barcoding has become an important tool for the accurate identification of raw medicinal plant materials used in herbal medicine and pharmaceutical preparations. Traditional identification methods based on morphology and microscopic characteristics may not always provide reliable results, particularly when plant materials are dried, fragmented, or processed. DNA barcoding overcomes these limitations by analyzing specific genetic sequences that remain stable regardless of environmental conditions or plant developmental stages. By comparing the obtained DNA sequences with reference databases, researchers can accurately determine the species identity of medicinal plant samples. This technique ensures that only authentic plant species are used in herbal formulations, thereby improving the reliability and therapeutic effectiveness of plant-based medicines.

Detection of Adulterants and Substitutes in Herbal Drugs

Adulteration and substitution are common issues in the herbal drug industry, often occurring due to economic motivations or misidentification during plant collection. DNA barcoding provides a powerful method for detecting adulterants by identifying genetic differences between authentic medicinal plants and substituted species. Even small quantities of adulterant plant material present in herbal products can be detected through molecular analysis. This capability is particularly useful for quality control in herbal medicines where visually similar plant species may be mixed unintentionally or intentionally. DNA-based authentication therefore helps maintain the purity, safety, and efficacy of herbal drug products.

Authentication of Processed Herbal Products

Many herbal products are marketed in the form of powders, capsules, extracts, or tablets, in which morphological features of the plant material are no longer visible. Under such conditions, conventional pharmacognostic techniques become ineffective for species identification. DNA barcoding offers a reliable approach for identifying plant species even in processed or powdered herbal materials. Short DNA fragments known as mini-barcodes can be amplified from degraded DNA present in processed products, allowing accurate identification of the plant species used. This application is particularly valuable for regulatory agencies and pharmaceutical industries seeking to verify the authenticity of commercial herbal formulations.

Quality Control in Herbal Pharmaceutical Industry

Quality assurance is essential for ensuring the safety and effectiveness of herbal medicines. DNA barcoding is increasingly incorporated into quality control protocols in the herbal pharmaceutical industry to confirm the authenticity of plant raw materials and finished products. Molecular authentication methods can detect contamination, substitution, and adulteration in herbal products before they reach the market. International organizations such as the World Health Organization recommend the use of advanced analytical methods for the quality control of medicinal plants. By integrating DNA barcoding with traditional pharmacognostic and phytochemical evaluation techniques, the herbal industry can achieve higher standards of product quality and consumer safety.

Conservation of Medicinal Plant Biodiversity

DNA barcoding also plays a significant role in the conservation of medicinal plant biodiversity. Many medicinal plant species are threatened due to overharvesting, habitat destruction, and illegal trade. Molecular identification techniques help in monitoring plant populations, identifying endangered species, and regulating the trade of medicinal plants. Accurate species identification supports conservation strategies by preventing the exploitation of rare or protected species and ensuring sustainable utilization of plant resources. DNA-based identification also assists researchers in studying genetic diversity within plant populations, which is important for conservation and breeding programs.

Identification of Endangered or Rare Medicinal Plants in Trade

Illegal trade of endangered medicinal plants poses a serious threat to biodiversity. DNA barcoding can help authorities identify plant species involved in international trade and detect the presence of protected species in herbal markets. For example, endangered medicinal plants such as Nardostachys jatamansi are sometimes traded illegally due to their high medicinal value. Molecular identification techniques help regulatory authorities verify the authenticity of plant materials and enforce conservation regulations effectively.

Table 4: Applications of DNA Barcoding in Medicinal Plant Authentication

Application Area	Description	Importance
Identification of raw medicinal plants	Determines the exact species of plant materials used in herbal medicines	Ensures authenticity of medicinal plants
Detection of adulterants	Identifies presence of substituted or mixed plant materials	Improves safety and product purity
Authentication of processed herbal products	Enables species identification in powders, extracts, or capsules	Useful when morphological features are lost
Quality control in herbal industry	Molecular verification of raw materials and finished herbal formulations	Enhances reliability and regulatory compliance
Biodiversity conservation	Helps identify and monitor medicinal plant species in natural habitats	Supports sustainable use of plant resources
Detection of endangered species in trade	Identifies protected medicinal plants in commercial markets	Assists in enforcing conservation laws

Table 5: Selected Case Studies of DNA Barcoding in Medicinal Plant Authentication

Medicinal Plant	Common Name	Adulterant/Substitute Detected	DNA Marker Used	Outcome
Panax ginseng	Ginseng	Other Panax species	ITS	Accurate species discrimination
Curcuma longa	Turmeric	Other Curcuma species	matK	Authentication of genuine turmeric
Withania somnifera	Ashwagandha	Morphologically similar species	ITS2	Identification of authentic plant material
Ginkgo biloba	Ginkgo	Mixed herbal components	rbcL	Verification of plant identity
Camellia sinensis	Green tea	Adulterant leaves	trnH-psbA	Detection of contamination
Nardostachys jatamansi	Jatamansi	Substituted plant materials	ITS	Identification of endangered species

LIMITATIONS AND CHALLENGES OF DNA BARCODING IN MEDICINAL PLANT AUTHENTICATION

Lack of a Universal Barcode for Plants

One of the major challenges in plant DNA barcoding is the absence of a single universal genetic marker that can accurately distinguish all plant species. Unlike animals, where the mitochondrial COI gene is widely accepted as a universal barcode, plants exhibit lower mutation rates in mitochondrial DNA, making it unsuitable for species identification. As a result, multiple genetic markers such as rbcL, matK, ITS, and trnH-psbA are often used together to achieve reliable identification. Although the combination of markers improves discrimination, it also increases the complexity and cost of molecular analysis. Therefore, researchers continue to explore new genomic regions that could provide better resolution for plant species identification.

Incomplete Reference Databases

DNA barcoding relies heavily on reference sequence databases for accurate species identification. If a particular plant species is not represented in available databases, it becomes difficult to confirm its identity through molecular comparison. Databases such as GenBank and Barcode of Life Data System contain large collections of genetic sequences, but coverage of many medicinal plants is still incomplete. In addition, some sequences in these databases may be incorrectly annotated due to taxonomic errors, which can lead to inaccurate identification results. Continuous efforts are therefore required to expand and curate reference libraries for medicinal plant species.

DNA Degradation in Processed Herbal Products

Another challenge associated with DNA barcoding is the degradation of DNA in processed herbal products. Manufacturing processes such as drying, heating, extraction, and grinding can damage DNA molecules and reduce the quantity of intact genetic material available for analysis. In highly processed herbal formulations, DNA fragments may become too small or degraded for successful amplification. Although techniques such as DNA mini-barcoding and next-generation sequencing have been developed to address this issue, DNA degradation remains a limitation in certain applications of molecular authentication.

Hybridization and Genetic Similarity

Closely related plant species may exhibit very similar DNA sequences in commonly used barcode regions. Hybridization events and evolutionary relationships between species can result in overlapping genetic characteristics, making it difficult to distinguish species using a single DNA marker. This issue is particularly common among species within the same genus. In such cases, multilocus barcoding approaches that combine several genetic markers are required to improve the accuracy of species discrimination.

Technical and Economic Constraints

Although DNA barcoding provides highly accurate results, its implementation requires specialized laboratory equipment, trained personnel, and financial resources. Molecular techniques such as DNA extraction, polymerase chain reaction (PCR), sequencing, and bioinformatic analysis may not be readily accessible in all laboratories, particularly in developing regions where herbal medicines are widely used. The cost of sequencing and data analysis may also limit the routine application of DNA barcoding for large-scale quality control. However, continuous advancements in sequencing technology are gradually reducing these barriers.

RECENT ADVANCES IN MOLECULAR AUTHENTICATION OF MEDICINAL PLANTS

Next-Generation Sequencing (NGS)

Recent technological advancements in molecular biology have introduced high-throughput sequencing methods that significantly enhance plant identification capabilities. Next-Generation Sequencing allows simultaneous sequencing of millions of DNA fragments, enabling comprehensive analysis of genetic material from complex herbal samples. NGS techniques provide higher resolution compared with conventional sequencing methods and allow researchers to analyze multiple barcode regions simultaneously.

DNA Metabarcoding

DNA metabarcoding is an advanced molecular technique that combines DNA barcoding with next-generation sequencing to identify multiple species present within a mixed biological sample. This approach is particularly useful for analyzing complex herbal formulations that contain multiple plant ingredients. By sequencing barcode regions from mixed DNA samples, metabarcoding can detect the presence of all plant species within a product, including adulterants or contaminants. This technique has become increasingly important for the authentication of commercial herbal medicines.

Genome Skimming and Whole Chloroplast Genome Sequencing

Genome skimming is another emerging technique that involves low-coverage sequencing of the entire genome to obtain high-copy genetic regions such as chloroplast genomes, mitochondrial DNA, and ribosomal DNA. Whole chloroplast genome sequencing provides extensive genetic information

that can significantly improve species discrimination among closely related plants. These genomic approaches provide a more comprehensive understanding of plant genetic diversity and are becoming valuable tools in molecular pharmacognosy research.

Integration with Other Analytical Techniques

Modern research increasingly combines molecular identification methods with other analytical approaches such as phytochemical profiling, metabolomics, and chemotaxonomy. This integrated strategy improves the reliability of medicinal plant authentication by providing complementary information about both genetic identity and chemical composition. Such multidisciplinary approaches are particularly valuable for ensuring the quality and safety of herbal medicines used in pharmaceutical and nutraceutical industries.

FUTURE PERSPECTIVES

The future of medicinal plant authentication is likely to involve greater integration of molecular technologies with traditional pharmacognostic methods. Expansion of global DNA barcode libraries for medicinal plants will enhance the accuracy of species identification and improve the reliability of molecular databases. Advances in sequencing technologies are expected to reduce costs and make molecular authentication more accessible for routine quality control in herbal industries.

Portable DNA sequencing devices and rapid molecular diagnostic tools may also enable on-site identification of medicinal plants in the field or during raw material procurement. Additionally, regulatory authorities may increasingly adopt DNA-based authentication methods as part of official standards for herbal drug quality control. The integration of molecular pharmacognosy with digital databases, bioinformatics, and artificial intelligence may further improve the monitoring of medicinal plant resources and support sustainable utilization of biodiversity.

CONCLUSION:

Accurate identification of medicinal plants is essential for ensuring the safety, efficacy, and quality of herbal medicines. Traditional pharmacognostic methods based on morphological, microscopic, and phytochemical characteristics provide valuable information but often face limitations when dealing with processed or closely related plant species. DNA barcoding has emerged as a powerful molecular tool that enables precise identification of medicinal plants using standardized genetic markers.

The application of DNA barcoding in medicinal plant authentication offers numerous advantages, including the detection of adulterants, verification of plant species in processed products, and support for quality control in the herbal pharmaceutical industry. Advances in molecular technologies such as next-generation sequencing, DNA metabarcoding, and genome skimming have further expanded the potential of molecular pharmacognosy.

Despite certain limitations such as incomplete reference databases and DNA degradation in processed samples, ongoing technological developments continue to improve the reliability and accessibility of DNA-based identification methods. Integrating molecular techniques with traditional pharmacognostic approaches will play a vital role in strengthening quality assurance systems for herbal medicines and promoting the safe and sustainable use of medicinal plant resources worldwide.

CONFLICT OF INTEREST:

Authors declare no any conflict of interest.

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