

# HERBAL MEDICINE IN INDIA: CONTEMPORARY STATUS AND MOLECULAR MARKER INTEGRATION IN HERBAL DRUG TECHNOLOGY

## Abstract

Herbal drugs play a significant role in traditional medicine practices, particularly in countries like India & China. In India, the traditional system of medicine known as Ayurveda relies heavily on the use of plants, animals, and minerals for the well-being of individuals. India being a hotspot of mega biodiversity, it is crucial to utilize medicinal plants in a rational and sustainable manner while ensuring the conservation of biodiversity. The Department of Biotechnology and the Indian Council of Agricultural Research have identified the top medicinal plants in India that are in high demand globally for import and export purposes. Scientific validation of the pharmacological activity of traditional drugs used in Ayurveda where medicinal plants are selected based on many centuries of experiences. DNA-based molecular markers are useful in many disciplines such as taxonomy, physiology, embryology and genetics, amongst others. DNA-based techniques have been extensively utilized for the authentication of medicinally important plant species. Aim of this book chapter to provide information on various aspects related to Indian herbal drugs and molecular markers use in herbal drug technology.

**Keywords:** Herbal Medicines, Medicinal Plants, Herbal Drug Technology, Molecular Markers.

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## I. INTRODUCTION

At the current rate of growth, the world's population will most certainly exceed 7.5 billion. The majority of this increase has occurred in undeveloped or impoverished countries, where about 80% of the population continues to rely on conventional systems and treatments based on herbal remedies. These folk or domestic treatments are easily accessible in the area, affordable, and have been time tested to be safe. The use of medicinal plants as medications, nutritional supplements (nutraceuticals), and cosmetics is increasing not just in impoverished countries but also in wealthy ones.[1]. Herbal remedies are described as "raw drugs of vegetable origin used for the treatment of diseases, frequently of a chronic nature, or to attain or maintain a state of improved health." By means of central metabolism, these plants produce organic substances known as "secondary metabolites" from primary metabolites. By interacting with the ecosystem, these metabolites not only significantly contribute to how plants adapt to their environment, but they are also widely prominently used in pharmaceuticals[2]. The raw or processed parts of a herb, including as the stems, flowers, roots, leaves, and seeds, can be used to make complex combinations of various organic components known as medicinal plant products. A safety efficacy is required for dietary supplements, like pharmaceutical medications, and medicinal plants are recognized as such under current regulations. Although it's common to believe that plants are "natural" and therefore harmless, several negative effects have been discovered as a result of active substances, pollutants, or medication interactions.[3]. Molecular markers are amplified bits of biological constituents such as primary and secondary metabolites, as well as other macromolecules such as nucleic acids. Adaptors are ligated to the ends of restriction fragments, followed by amplification with adaptor homologous primers. AFLP can detect hundreds of distinct loci and may be used to DNAs of any origin or complexity[4].

## II. CURRENT TRENDS IN HERBAL MEDICINE

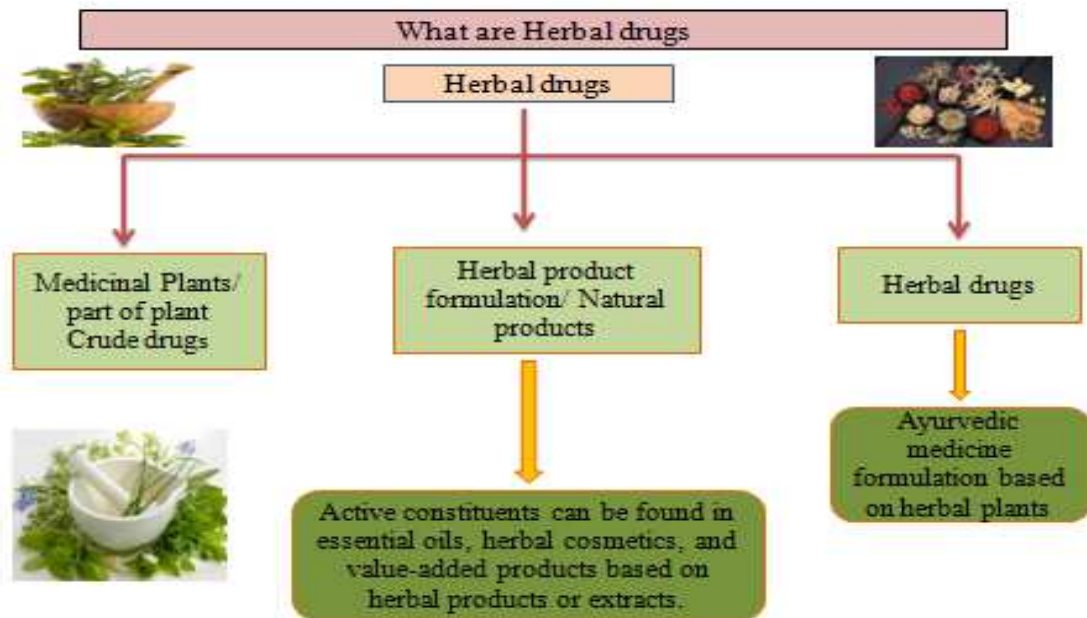
Plant research has been a focus in India for many years. A large range of plants have been studied for their pharmacological properties. Herbal preparations that have gained widespread acceptance as therapeutic treatments include nootropics, anti-hypertensives, hepatoprotective drugs, anti-inflammatory agents, antidiabetics and cholesterol lowering medicines. Some of them have undergone extensive experimental and clinical investigations[5][6].

The Drugs Directorate has issued rules for the manufacturing and marketing of three types of botanical products:

- Nutritional supplements (no DIN necessary, no therapeutic claims)
- Phytopharmaceuticals having complete drug status (authorized therapeutic indications, acceptable dose, scientific evidence of efficacy, DIN needed)
- Traditional herbal remedies (only for self-medication, efficacy backed by herbal literature, accepted therapeutic indications and dose) (Leung, 1980) [7][8][9]

**1. Herbal Medicine Scenario in India:** In India, the first National Health Policy, issued in 1983, states that India's rich legacy of health care should be included into national programs. The AYUSH department, which was established in 1995, oversees traditional

medicine (TM) programs. Since 2002, the Indian government has had a specific and independent policy on TM, which is likewise governed under the Drugs and Cosmetics Act of 1940. GMPs have been required since 2002, but not exactly as suggested by WHO, but they are nonetheless based on them. The central and state governments are putting pressure on industrial firms to conform with GMP norms and maintain quality standards. The global market for herbal medicine, comprising herbal products and raw materials, is expected to develop at a 5 to 15% annual pace [7][8][9]. A significant portion (60%) of the herbal medicine exports consists of psyllium seeds and husk, castor oil, and opium extract. These products dominate the export market, while other herbal formulations seem to have a smaller presence internationally. Despite a relatively significant volume of exports, the profitability is minimal. This is likely due to the fact that around 80% of the country's exports to industrialized nations are basic pharmaceuticals rather than completed formulations. Basic pharmaceuticals may have lower profit margins compared to value-added finished products[10]. India has a long-standing history and traditional knowledge of herbal medicine, particularly in ayurveda, unani, and siddha systems. These systems offer a diverse range of herbal formulations with potential global demand. The herbal medicine market in industrialized nations may pose challenges for Indian exporters. These challenges could include stringent regulatory requirements, quality standards, competition from other countries, and the need for research and validation of traditional remedies[7]. To enhance the export potential of herbal medicines, India could focus on the following strategies. Instead of exporting basic pharmaceuticals, there should be a shift towards exporting value-added finished herbal formulations. This can increase profitability and create more demand in international markets[11]. Emphasizing quality control measures, conducting research to validate traditional remedies, and adhering to international standards will build trust among consumers and regulators in importing countries. Promote the benefits of Indian herbal medicines, especially the unique formulations rooted in traditional knowledge, in order to capture new markets and consumers worldwide[12]. Reducing the dependence on a few major products for exports and diversifying the range of herbal medicines can help expand the market reach and mitigate risks. Provide support and incentives to small and medium-sized enterprises (SMEs) engaged in herbal medicine production to encourage innovation and entrepreneurship in this sector. Collaborations between the government, private sector, and research institutions can lead to the development of high-quality herbal products with wider acceptance in international markets. By addressing these challenges and adopting appropriate strategies, India can leverage its rich heritage of herbal medicine to make a more significant impact on the global market[13].



**Figure 1:** Herbal Drugs



**Figure 2:** Advantages of Herbal Medicines

## 2. Some Herbal Plants use in Herbal Medicines

S.No.	Herbal drugs	Botanical Name / Family	Chemical Constituents	Herbal Uses
1	Garlic	<i>Allium sativum</i> / Liliaceae	It contains S-allylcysteine fructosyl arginine, Saponins S-allyl mercaptocysteine[14][15]	Characteristic pungent, spicy flavor that mellows[16]
2	Ginger	<i>Zingiber officinale</i> /Zingiberaceae	Constituents $\beta$ -phelladrene, cineol, citral shogaols gingerols, $\beta$ -sesquiphellandrene bisabolene, farnesene[17]	It used as spice, ginger tea cookies, crackers, cakes[18]
3	Turmeric	<i>Curcuma longa</i> /Zingiberaceae	Contains, curcumin, demethoxycurcumin, turmerone, Curcuminoids,	It is used to color,enhance the flavors of certain dishes[20]
4	Brahmi	<i>Bacopa monnieri</i> /Scrophulariaceae	It contains Stigmastanol, b-Sitosterol, Betulinic acid, D-Mannitol[21]	Treatment of epilepsy, memory[22]
5	Aloes	<i>Aloe vera</i> / Liliaceae	It contains Aloe-emodin, aloetic-acid,Arachidonic acid, campesterol[23][24]	Used as a food product,beverages, cosmetics products[25][26]
6	Asafoetida	<i>Ferula asa fetida</i> /Umbelliferae	Contains ferulic acid, volatile oils, Resin, Gums, umbelliferone[27]	Used as flavouring agents, digestion , heart diseases treatment[28]
7	Bael	<i>Aegle marmelos</i> /Rutaceae	Contains lupeol, cineol, citral,eugenol[29]	Use in Bela pana in refreshing drink as a juice, make a drink similarlemonade[30]
8	St.john'swort	<i>Hypericum perforatum</i> / Hypericaceae	Contains epigallocatechin, rutin mentoflavone, astilbin, uercitrin[31]	Remedy for wound and muscle pain, use in premenstrual syndrome[32]

9	Valeriana	<i>Valeriana officinalis</i> / Valerianaceae	Contains valerenic acid, beta-sitosterol, ursolic acid, caryophyllene acid[33]	Used in teas and capsules[34]
10	Ginseng	<i>Panax ginseng</i> / Araliaceae	It contains prosapgenin, and ginsenoside[35]	Energy drinks or hair tonics [36]
11	Green tea	<i>Camellia sinesis</i> / Theaceae	It is a powerful agents use as antioxidants [37]	It uses a antioxidants and protection use as some cosmetics[38]
12	Buch-Hum	<i>Crataeva murula</i> / Capparidaceae	It contains lupeol and its acetate, ceryl, diosgenin and betulinic acid[39]	Used in gall bladder and kidney stone, fever, vomiting[40]
13	Eucalyptol	<i>Eucalyptus globules</i> /Myrtaceae	Sabinene and alpha-Terpinyl acetate, a-Pinene, trans-/beta osimen [41]	Insecticide and insect repellent, cough suppressant, Mouthwash[42]
14	Witch Hazel	<i>Hamamelis virginiana</i> / Hamamelidaceae	Hamamelitannins, gallic acid, catechins, safrole[43]	Treatment of acne, facial care products make use of the astringent properties[44]
15	Chamomile	<i>Matricaria chamomilla</i> / Asteraceae	Quercimeritrin, Rutin, luteolin, apiin, apigenin, apigetrin[45]	Antigenotoxic, antihyperglycemic, anti-inflammatory[46]

### III. HERBAL DRUG TECHNOLOGY

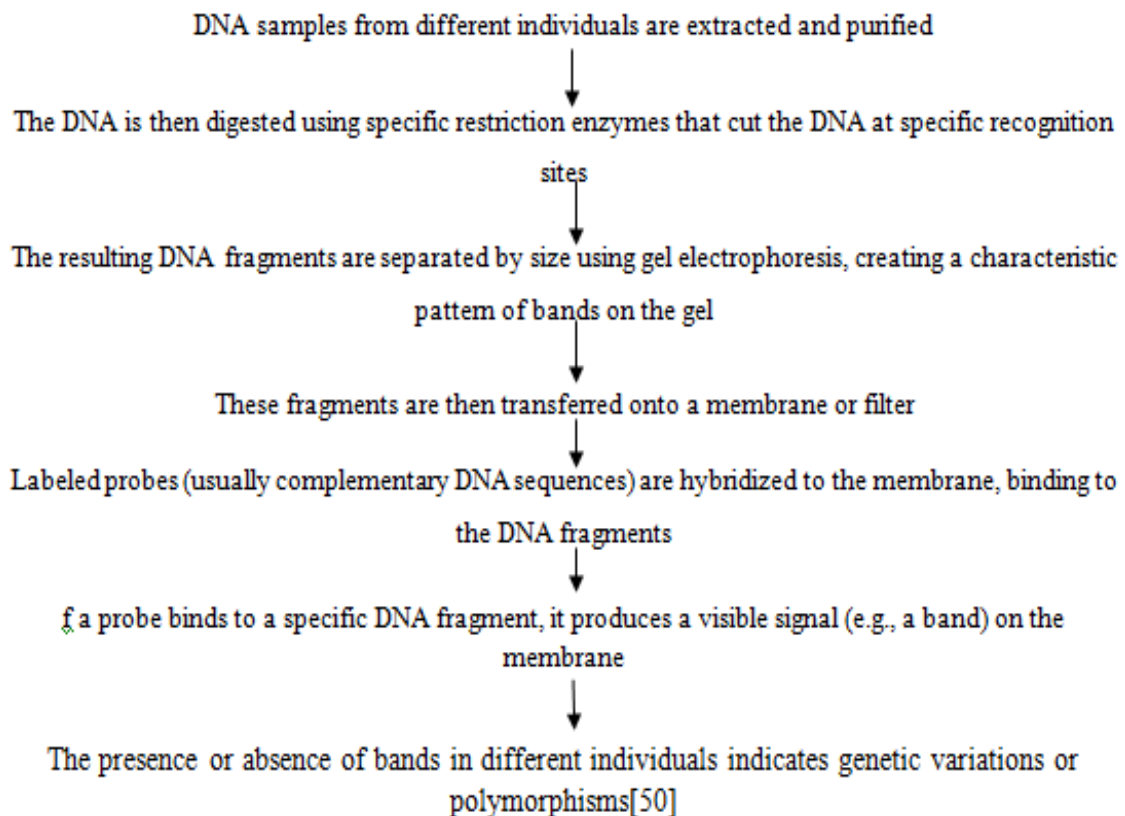
Herbal medication technology is used to turn botanical resources into medicines, where standardization and quality control are essential, as is effective integration of current scientific procedures and traditional knowledge[47]. The use of chromatographic methods and marker chemicals to standardize botanical preparations has limits due to their various origins and chemical complexity. DNA-based molecular markers are useful in domains such as taxonomy, physiology, embryology, genetics, and so on. DNA-based approaches have been frequently utilized to authenticate plant species of therapeutic relevance. Pharmacognosy primarily handles quality-related concerns by utilizing standard botanical and organoleptic characteristics of crude pharmaceuticals, as well as chemo profiling aided characterization with chromatographic and spectroscopic methods. The new pharmacognosy encompasses all elements of drug research and discovery in which biotechnology-driven applications play a key role. The current focus is on chemotype-driven fingerprinting[48][49].

### IV. TYPES OF DNA MARKERS USED IN HERBAL DRUG TECHNOLOGY

#### 1. Hybridization- Based Marker

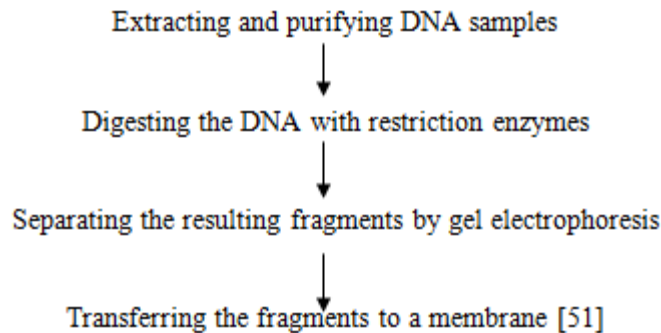
- **Restriction Fragment Length Polymorphism (RFLP):** RFLP is a technique that exploits variations in the lengths of DNA fragments resulting from the use of restriction enzymes.

##### Workflow of RFLP marker



- **Variable Number Tandem Repeats (VNTR):** VNTRs are repetitive DNA sequences that vary in the number of tandem repeats between individuals. The VNTR analysis technique focuses on detecting these variations. Hybridizing labeled probes that target the VNTR regions, revealing the presence or absence of specific VNTR alleles.

#### Work flow of VNTR marker



In both RFLP and VNTR analysis, the hybridization of labeled probes to DNA fragments allows researchers to identify polymorphisms based on the patterns of bands or signals that appear on the membrane or gel[52]. These techniques were widely used in the past for DNA fingerprinting, paternity testing, and studying genetic variations. However, newer and more advanced methods, such as PCR-based techniques and DNA sequencing, have largely replaced these methods due to their higher sensitivity, specificity, and efficiency[53].

2. **PCR-based Method:** PCR-based markers, also known as PCR markers or molecular markers, are segments of DNA that are used in Polymerase Chain Reaction (PCR) reactions to identify specific genetic traits or variations within an individual or a population [54]. PCR is a laboratory technique used to amplify a specific DNA segment, making it easier to study and analyze. PCR-based markers are widely used in various fields of biology, including genetics, genomics, plant breeding, and forensic science, among others. They provide valuable information about genetic diversity, inheritance patterns, and the presence of specific genes or alleles. There are several types of PCR-based markers, each with its own applications and advantages[55]. Some of the common types includes:

- **Single Nucleotide Polymorphism (SNP) Markers:** These markers focus on single nucleotide variations within DNA sequences. SNPs are the most abundant type of genetic variation in populations and can be used to study diseases, population genetics, and individual traits[56]. A single nucleotide polymorphism (SNP) is a DNA sequence variation that occurs when a single nucleotide (A, T, C, or G) in the genome differs among individuals or populations. This variation can be found in coding or non-coding regions of the genome. SNPs are abundant in genomes and are present at a relatively high frequency in populations. They are estimated to occur approximately every 1,000 to 2,000 nucleotides in the human genome[57].



- **Simple Sequence Repeat (SSR) Markers:** SSR markers are short DNA sequences in which a unit of 1-6 nucleotides is repeated multiple times in tandem. For example, a common SSR motif might be "AGAGAGAGAGAG" The number of repeats in an SSR can vary between individuals, resulting in length polymorphisms that serve as genetic markers. SSRs are abundant throughout genomes and can be found in both coding and non-coding regions. Due to their high mutation rates, they can rapidly accumulate variations in repeat numbers over generations.

Also known as microsatellites, SSRs are short DNA sequences that are repeated in tandem. These markers are particularly useful for studying genetic diversity and relationships among closely related species or populations[58][59].

- **Amplified Fragment Length Polymorphism (AFLP) Markers:** AFLP involves the selective amplification of a subset of genomic DNA fragments using PCR . It is a PCR-based method that combines the specificity of restriction enzymes with the sensitivity of PCR amplification [60].

**Procedure:**

- **Digestion :** Genomic DNA is digested with two different restriction enzymes to create fragments. One of the enzymes has a selective site and is usually 4-base cutter, while the other is a frequent cutter.
  - **Ligation and Pre-amplification :** Adapters containing specific sequences are ligated to the ends of the restriction fragments. These adapters contain primer-binding sites for PCR amplification. A pre-amplification step using primers complementary to the adapter sequences is performed to generate a pool of DNA fragments with adapters.
  - **Selective Amplification :** Selective amplification involves using a subset of primers that have one or more selective nucleotides at the 3' end. These selective nucleotides correspond to specific bases adjacent to the restriction site. This step amplifies a subset of fragments with specific sequences.
  - **Gel Electrophoresis :** The amplified fragments are separated by size using gel electrophoresis. The resulting pattern of bands represents the AFLP profile AFLP markers are a valuable tool in genetic research, offering insights into genetic diversity and relationships. The technique's ability to generate a large number of markers without prior sequence information makes it useful for studying various organisms, from plants to animals. This technique is versatile and has applications in population genetics and phylogenetic studies[61][62].
- **Random Amplification of Polymorphic DNA (RAPD) Markers:** Random Amplification of Polymorphic DNA (RAPD) markers are a molecular technique used in genetics and genomics to detect genetic variation among individuals or populations. RAPD is a PCR-based method that involves the amplification of random DNA segments using short, single arbitrary primers. It is a simple and cost-effective

technique that has applications in various areas of genetic research. Here's an overview of RAPD markers [63].

**Procedure:**

- **Primer Selection:** A set of short, single arbitrary primers is selected. These primers are typically 10-20 nucleotides in length and have arbitrary sequences.
- **Amplification:** Genomic DNA is subjected to PCR using a single arbitrary primer. The primer anneals to complementary sequences in the DNA, and DNA fragments are amplified using the PCR process.
- **Gel Electrophoresis:** The amplified DNA fragments are separated by size using gel electrophoresis. The resulting pattern of bands represents the RAPD profile.
- **Polymorphism Detection:** RAPD markers are detected based on differences in the presence or absence of amplified bands among individuals or populations. The banding pattern reflects genetic variation.

RAPD is a relatively simple and cost-effective technique that does not require prior sequence information. It can be applied to organisms with limited genomic information or in cases where specific markers are unavailable. RAPD markers are a versatile tool for detecting genetic variation, especially in cases where specific markers are not available. While RAPD has limitations, it has been widely used in genetic research to study genetic diversity, population structures, and evolutionary relationships across a variety of organisms [64][65][66].

- **Sequence Characterized Amplified Region (SCAR) Markers:** Sequence Characterized Amplified Region (SCAR) markers are a type of molecular marker used in genetics and genomics research. SCAR markers are derived from specific DNA sequences that are associated with particular traits or regions of interest. These markers are developed through the conversion of random amplified polymorphic DNA (RAPD) or amplified fragment length polymorphism (AFLP) bands into reliable and reproducible PCR-based markers. SCAR markers offer greater specificity and consistency compared to RAPD or AFLP markers, making them valuable tools in various genetic applications [67][68].

**Development of SCAR**

- **Initial Amplification:** RAPD or AFLP analysis generates numerous DNA fragments, some of which may show polymorphism between different individuals or populations.
- **Cloning and Sequencing:** Bands of interest are extracted from the gel, cloned into vectors, and sequenced to identify the DNA sequence responsible for the polymorphism.

- **Primer Design:** Based on the sequenced DNA, specific primers are designed that will amplify a targeted region in a reproducible manner.
- **Testing and Validation:** The newly designed primers are tested to confirm their ability to amplify the desired DNA fragment consistently across different samples.

PCR-based markers have revolutionized many areas of biology by enabling researchers to study genetic variation in a targeted and efficient manner. They have applications in agriculture, medicine, ecology, and more. These markers provide insights into evolutionary relationships, disease susceptibility, population dynamics and even individual identity in forensic contexts[69][70].

**3. Sequencing-based Marker:** DNA sequencing has revolutionized our ability to identify and classify species. The variations you mentioned, such as transversions, insertions, deletions, and single nucleotide polymorphisms (SNPs), provide a wealth of genetic information that can be used for species identification and phylogenetic studies[71].

Here are some key points about using DNA sequencing for species identification and authentication:

- **Single Nucleotide Polymorphisms (SNPs):** SNPs are variations at the single nucleotide level that can occur between individuals of the same species. These variations are often used as genetic markers for species identification. High-throughput sequencing techniques have made it feasible to identify and analyze large numbers of SNPs in various organisms, providing a powerful tool for classification.
- **Barcode Sequences:** Some genomic regions are used as "barcodes" for species identification. These are short DNA sequences that are highly variable between species but relatively conserved within a species. The DNA barcode approach allows rapid and accurate species identification, even for specimens that might be difficult to identify using traditional methods.
- **Chloroplast and Ribosomal DNA Sequences:** Sequencing regions of chloroplast DNA (e.g., trnK) and ribosomal DNA (e.g., ITS) has proven particularly useful for identifying plant species. These regions have varying levels of variation depending on taxonomic levels and can offer insights into evolutionary relationships.
- **Species Identification:** DNA sequencing allows us to directly analyze the genetic code of an organism. By comparing the sequences of specific genes or genomic regions across different individuals, scientists can determine the level of genetic similarity and relatedness. This information can be used to identify species and differentiate between closely related organisms.
- **Phylogenetic Studies:** Phylogenetic studies aim to reconstruct the evolutionary relationships between different species or groups of organisms. DNA sequencing, especially of specific marker genes like the 18S-26S ribosomal DNA (rDNA) and

internal transcribed spacer (ITS) regions, can reveal the genetic distances and branching patterns that indicate evolutionary relatedness.

- **Authentication and Conservation:** DNA sequencing is crucial for verifying the authenticity of biological specimens and products, especially in fields like forensics, food industry, and conservation. By analyzing specific DNA markers, scientists can confirm the identity of species and detect instances of mislabeling or fraud.
- **Advancements in Sequencing Technology:** Rapid advancements in DNA sequencing technologies, such as next-generation sequencing (NGS) and now even more advanced techniques have made it possible to obtain vast amounts of genomic data quickly and at a lower cost. This has significantly expanded the scope and accuracy of species identification and phylogenetic analysis.

In summary, DNA sequencing, especially of specific genomic regions, has transformed the field of species identification and phylogenetics. It provides a definitive and accurate means of determining species relationships and authenticity with applications spanning diverse scientific disciplines and industries[72][73][74][75].

## V. APPLICATIONS OF MOLECULAR MARKERS IN HERBAL DRUG TECHNOLOGY

Molecular markers have revolutionized various fields, including herbal drug technology by enabling more efficient identification, authentication, quality control, and standardization of herbal medicines. Here are some applications of molecular markers in herbal drug technology[76][77][77].

1. **Plant Authentication and Species Identification:** Molecular markers such as DNA barcoding allow rapid and accurate identification of plant species used in herbal medicines. This helps prevent the use of misidentified or adulterated plant materials, ensuring the safety and efficacy of herbal products [79][80].
2. **Genetic Diversity Assessment:** Molecular markers help assess the genetic diversity within a plant species, which is crucial for breeding programs and conservation efforts. Understanding genetic variability can aid in selecting plant varieties with desirable traits, such as higher medicinal compound content[81][82][83].
3. **Quality Control and Standardization:** Molecular markers can be used to determine the presence and concentration of specific bioactive compounds in herbal medicines[84][85]. This information ensures consistent quality, potency, and efficacy of herbal products, which is vital for consumer safety and regulatory compliance[86][87].
4. **Detection of Adulteration:** Adulteration of herbal drugs with cheaper or ineffective plant materials is a significant concern. Molecular markers can help identify such adulterants, even in processed or powdered forms, thereby preventing fraudulent practices and ensuring product integrity[88][89].

5. **Pharmacological Studies:** Molecular markers can be linked to specific pharmacological activities or medicinal properties of plants[90]. researchers to identify and isolate bioactive compounds efficiently, leading to the development of new herbal drugs or formulations with targeted therapeutic effects[91][92].
6. **Tracing the Geographic Origin:** Molecular markers can be used to trace the geographic origin of herbal raw materials. This information can be valuable for marketing and promoting the authenticity of herbal products from specific regions known for their medicinal plants[93][94].
7. **Crop Improvement and Breeding:** Molecular markers aid in marker-assisted selection (MAS) in herbal plants[95]. This technique helps breeders identify and select plants with desired traits, such as increased yield, disease resistance, or improved medicinal compound content, accelerating the breeding process[96].
8. **Process Optimization and Cultivation Practices:** Molecular markers can be used to monitor the expression of genes involved in the biosynthesis of bioactive compounds. This knowledge allows the optimization of cultivation practices to enhance the yield and quality of herbal medicines[97][98].
9. **Conservation and Sustainable Utilization:** Molecular markers provide insights into the genetic diversity and population structure of medicinal plants. This information supports conservation efforts and sustainable utilization practices to prevent overexploitation and maintain the natural resources of medicinal plants[99][100].

In conclusion, molecular markers have a wide range of applications in herbal drug technology, improving the safety, quality and efficacy of herbal medicines while facilitating their sustainable use and development.

## VI. CONCLUSION

In conclusion, herbal drugs and herbal drug technology represent a dynamic intersection of traditional wisdom and modern science. The rich history of medicinal herbs as sources of therapeutic assistance has paved the way for their integration into diverse healthcare systems around the world. These botanical treasures not only offer potential remedies for various ailments but also hold promise as contributors to overall health maintenance. As the global demand for herbal products continues to rise, it becomes imperative to ensure that the quality, safety and efficacy of these products are upheld. Robust quality control practices and standardized labeling are vital components in building consumer trust and promoting responsible usage. In the realm of agricultural production, the significance of both conventional plant breeding and biotechnology cannot be underestimated. While DNA marker technology and biotechnological advancements offer innovative tools to address breeding challenges, the enduring contributions of traditional plant breeding methodologies, responsible for more than half of global agricultural output must be acknowledged and valued.

In the journey ahead, the potential of herbal drugs to enrich global health landscapes remains undeniable. By embracing the lessons of the past, harnessing the power of present

technologies, and fostering a culture of innovation, the field of herbal drug technology is poised to contribute profoundly to the betterment of individuals and societies on a global scale.

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