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Review Article

A review on molecular techniques employed for authentication of Indian medicinal plants

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Abstract

Traditional medical systems are advancing to the level of modern medicines in treatment and preventive aspects. The increased trade in medicinal plants provides income source for herbalists while substitution of rare ingredients with cheaper and more readily available species is misleading the end users. The prime cause of the problems associated with the standardization of medicinal plants is complex composition of herbal drugs used in the form of whole plants, plant parts or extracts. Deliberate adulteration of intended ingredients are posing difficulty in distinguishing the genuine resources. Authentication of medicinal plants by recent molecular techniques is inevitable for herbal drug industries, researchers and academia. Of late, herbal genomics, molecular studies of medicinal plants and powerful next generation sequencing techniques have been emerged to transform the current knowledge. A compilation of various molecular markers used, their efficiency in barcoding for the purpose of accurate authentication of herbal drugs has been attempted in this study. Data were collected from previous literature and online repositories like NCBI, Pubmed etc. There are various molecular techniques that can be exploited for authentication of medicinal plants such as Restriction Fragment Length Polymorphism (RFLP), Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), Sequence Characterized Amplified Region (SCAR), Selective Amplification of Microsatellite polymorphic loci (SAMPL), Simple Sequence Repeats (SSR), Inter Simple Sequence Repeat (ISSR), DNA barcoding, Next Generation Sequencing Techniques etc. Some of medicinal plants were reported having molecular data useful in plant identification. The genomic data of poly herbal formulations helps for scientific validation and universal recognition. Even though the challenges associated with reprehensibility, primer designing, amplification products of molecular markers and troubles related with DNA isolation and purification, become the major obstacle in front of researchers. It is high time to focus these novel strategies for proper identification to ensure the fidelity of traditional herbal products and there by promoting a step towards the global acceptance of our indigenous medicinal systems.

Keywords: Adulteration; RAPD; RFLP; AFLP; SCAR; Plant barcoding

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Introduction

Indian flora is enriched with 3000 to 3500 species of medicinal plants used in traditional systems of medicine in which 2500 species are endemic (1). India is being blessed with its own systems of medicine originated in ancient times in connection with particular culture and geographical locations which are based mostly on the indigenous herbal plants available. Since the plants having different

names locally the chance of misidentification is more and will affect the quality of it. In this regard plants are to be authenticated by eminent botanists if in fresh form or by pharmacognostical and by chemical finger printing methods when in dried form. Conventional macro-microscopic examinations and other quality assurance tools do not aid in critically distinguishing raw materials derived from closely related species, adulterants or substitutes (2). According to the WHO general guidelines for methodologies on research and evaluation of traditional medicines, first step in assuring quality, safety and efficacy of traditional medicines is correct identification (3).

Standardization of medicinal plants is necessary for its authentication resulting in botanical identity. The quality control and identification of herbal plants can be done through the analysis of well-defined marker compounds. However, in many herbal species the chemical composition of the plant changes with the external environment and processing conditions, which lowers the reliability of these authentication methods (4). Molecular techniques helps to overcome all these drawbacks associated with other methods. Now some of the research centers in India have started molecular profiling to focus on the genetic information of both fauna and floras. The researchers in India have tried to spot convinced easy and modern molecular biology technologies that can be employed in order to identify the function of the genes and their higher usability of ancient knowledge of medicinal plants to treat the human diseases by identifying the effect of the bioactive compound(s) in medicinal plant(s) to treat patients and to enhance conventional treatment for better patient outcome (5). As India is diversified with its resource of biodiversity, so many molecular studies and genetic data has been recorded as part of conservation and to manage the diversity as well as keeping awareness to the public about our vast precious bio-resources. Owing to the large scale inventorization of biodiversity conservation DNA barcoding of tropical trees of India were done using ITS and trnH-psbA and considered to be highly successful (6). DNA barcoding has been used to discriminate at species level and it is helped to trace out a new cryptic grass species in an ethnobotanic study by the hill tribes of the Western Ghats in South India (7).

This review provides insight on need for molecular authentication of medicinal plants to improve the quality, safety and efficacy of the drugs and also gives a brief account of the most commonly used DNA-based technologies (RAPD, RFLP, AFLP, SCAR, sequencing, microarrays, next generation sequencing techniques) including suitable examples of South Indian medicinal plants.

Data aquisition

Extensive literature review was carried out by refereeing various books, monographs, articles and journals along with online portals (NCBI, PUBMED) pertaining to medicinal plants and their recent molecular studies.

Results

Authentication of medicinal plants by molecular markers

DNA markers which are based on distinct genetic organization are having a greater advantage over other marker systems. These markers are not tissue specific and thus can be detected at any stage of plant development. As the information generated by this technology can be easily automated, it gives accurate and efficient methods which will be cheaper and more authentic than the phenotypic and chemical markers (8).

A genetic marker or DNA markers are the unique DNA sequences which can be used in DNA hybridization, PCR or restriction mapping experiments to identify target sequence. It gives the direct reflection of genotype (9).

Molecular markers used for authentication

***DNA hybridization based markers* (*Non PCR based markers*):** It is widely used method for identification purposes. The limitation of this approach is that it needs high quantity of DNA and use of radio labeled probes for experiments (9).

***RFLP* (*Restriction Fragment Length Polymorphism*)**

It is a molecular marker used for the separation and identification of desired fragments of DNA using restriction enzymes. It is the first used technology employed for detection of polymorphism based on DNA differences. It involves the isolation of DNA, itsdigestion by restriction enzymes and the fragments separation by gel electrophoresis. The desired fragment is detected by using labeled probes. The main limitation of this technique is, it requires large amount of sample DNA and it is time consuming and labor intensive (9).

Markers based on PCR amplification: PCR technique is used for the amplification of desired DNA sequences. It requires low quantity of DNA for the experiments (9).

RAPD (Random Amplification of polymorphic DNA)

It is a non-locus specific DNA marker used for the amplification of desired DNA fragments for the detection of polymorphisms based on PCR amplification using short synthetic primers. The amplified products are separated by electrophoresis and detected. It is very quick and easy technique and there is no need of sequence

data for primer construction. The RAPD have high genomic abundance and is found randomly distributed throughout the genome but it is low reproducible and unsuitable marker for comparison of similar species (9).

AFLP (Amplified Fragment Length Polymorphism)

This is also a non-locus specific DNA marker employed for identification purposes which involves detection of genomic restriction fragments and can be used for DNAs of any origin or complexity. AFLP is based on the principle of generation of DNA fragments using restriction enzymes and oligonucleotide adaptors (or linkers), and their amplification by PCR. Thus, this technique combines the usefulness of restriction digestion and PCR. It is very sensitive and reproducible (9).

SCAR (Sequence characterized amplified region)

In this technique 18 to 25 base pair primers, based on a unique RAPD sequences are used. It is possible to perform SCAR under high stringent reaction conditions enhancing its specificity and stability (8). These primers are usually much longer than RAPD primers and allow for PCR analysis; therefore, their amplicons are more reproducible and easily recognized (10).

Microsatellite based molecular markers

Some of most popularly used microsatellite markers are discussed below:

SSR (Simple Sequence Repeats) markers

These are the most efficient markers of 2 to 5 DNA base pairs and are a type of variable number tandem repeats (VNTRs). SSRs are co-dominant molecular markers that distinguish homozygotic and hetrozygotic individuals and also possess a large number of alleles. In fact, the use of single SSR marker may not provide authentic information hence we have to use different SSR markers for reliable and accurate differentiation of plants (11).

ISSR (Inter Simple Sequence Repeat) markers

PCR based technique reported by Zietkiewicz 1994, (12) involves amplification of DNA segments between two identical microsatellite repeat regions oriented in opposite direction using primers designed from microsatellite core regions. The technique uses microsatellite primers, usually 16 to 25 base pairs long. ISSR technique is simple, quick and less costly like the RAPD technique. ISSR markers have high reproducibility than RAPD primers due to the longer primer length (13).

SAMPL (Selective Amplification of Microsatellite polymorphic loci) markers

This is a microsatellite-based dominant marker system which is a modification of AFLP techniques (14). DNA is prepared in the same way as for an AFLP assay, allowing the use of the same pre-amplified samples, but changing the primer used in the selective amplification for a microsatellite sequence (15).

DNA barcoding

This involves the application of short DNA sequences for identification of organisms. In DNA barcoding the main focus is to find out a universal DNA sequence that must a balance of conserved sequence as well as harbor enough diversity in order to differentiate organisms (8). Many chloroplast genomic regions (rbcL, matK, trnH-psbA, trnL-F, rpl36-rps8, ITS and 5S rRNA) have been evaluated in plant systems by- "The Consortium for the Barcode of Life Plant Working Group (CBOL)"(16) for making barcodes for better identification of plants. Cameron and Chase 1999, (17) showed less species discriminating ability with rbcl and matK when very close taxa are concerned. Hence, Li *et. al.*, 2011, (18) included nuclear ITS to the combination matK + rbcL with the aim to have better discriminating ability in closely related species.

Next generation sequencing technologies

It is a well advanced and powerful DNA sequencing method for whole genome representation, which aids in complete characterization and analysis of genetic and genomic resources. Most NGS methods are based on polymerase chain reaction (PCR) amplification of platform-specific DNA fragment libraries, which are then sequenced (19). This new technology will transform the biological world to be astonished one which we cannot even imagine. This technology can bring vast changes in the medical field since it is able to perceive the all aspects of genomic alterations which lead to diseases like cancer. NGS provides platforms for the study of exome, transcriptome, epigenome and whole genome. It needs time investment, advanced laboratory facilities and involves computational analysis and bioinformatics studies.

These methods can mainly divided to three types: sequencing by synthesis, sequencing by ligation, and single-molecule sequencing.

Sequencing by synthesis: Here, the appropriate DNA fragment is ligated to adaptor sequences and amplified to improve the fluorescent or chemical signal. Templates are immobilized in preparation for flow-cell cycles after separation (20).

Sequencing by ligation: Different lengths of florescent labelled oligonucleotide probes are used in this method. Anchor sequences which used as primers help for hybridization and DNA ligase in the flow cell attach probes to the primer and template. The incorporated probe is determined by fluorescence imaging (20).

Single-molecule sequencing:

It is known as third generation sequencing which overcomes the difficulties of other NGS technologies. This method employs use of single nucleic acid molecule for DNA sequencing which excludes the DNA template amplification. Here, the incorporation of nucleotide is detected as a signal by chemi-luminescence. This technique is more advanced and having high multiplex ability (20). Various Next-Generation Sequencing (NGS) Platforms used are listed in Table 1 (20, 21).

Plant barcoding system

Molecular taxonomists now envisage cataloging all living species on earth using so-called DNA barcodes, the nucleotide sequence of a short DNA fragment (22-24). In the past two decades the molecular investigations of systematic problems have progressed from uncommon curiosities to a standard means of elucidating phylogenetic history (25). The use of genome-based methods for the authentication of medicinal plants should be seen in the context of plant phylogenetic studies and a general effort aimed at barcoding of all plants (26, 27). A preliminary system for DNA barcoding herbal materials has been established based on a two-locus combination of ITS2 + *psbA–trnH* barcodes. There are 78,847 sequences belonging to 23,262 species in the system, which include more than 95% of crude herbal drugs in pharmacopeia, such as those of China, Japan, Korea, India, USA and Europe (28). Although there are no specified DNA sequences for plant barcoding, the psbA-trnH spacer region has been tested widely and found to be effective (29).

Role of Next generation Technologies in transforming the knowledge of biological world

NGS technologies have emerged as a flagging leader in the modern era of scientific discoveries. Every branch of biological science has become the vital part of genomics since these methods have paved the most easily, accessible and cost effective genome sequencing. The researchers are discovering new approaches and seeking novel interdisciplinary branches for the application of NGS. Few studies are reported in plants in which NGS technologies applied for molecular characterization. In a study (30), the authenticity of NGS for developing SSR in plants via their work on Cranberry was convinced and the authors reviewed 95 other studies for the same by Sanger, Illumina and 454 technologies. In the case of species that do not having a reference genome, the NGS-based SNP discovery is very challenging (20). There are studies where usage of four short read alignment tools (Maq, BowTie, Novoalign, and SOAP2) using their novel strategy called coverage-based consensus calling (CbCC) for SNP discovery as a case study in chickpea, *Cicer arietienum* L., a crop lacking a reference genome. They found Maq as effective technique (31).

Table 1. Various Next-Generation Sequencing (NGS) Platforms (20, 21)

	Platform	Library	NGS chemistry	Advantages
Sequencing by synthesis	**Roche/454 GS FLX+**	Fragment, Mate-pair/Emulsion PCR	Pyrosequencing	Longer reads, fast run times; good choice for de novo assembly
	Illumina HiSEq. 2000	Fragment, Mate-pair, solid-phase	Reversible Terminators	Currently most widely used platform, high coverage
	Ion TorrentPGM	Fragment, Emulsion PCR	Natural nucleotides 200	Very fast run time, cost effective, open source
Sequencing by Ligation	**Life/AB SOLiD5500 Series**	Fragment, Mate-pair /Emulsion PCR	Cleavable probe Sequence by ligation	2-Base encoding error correction
	PolonatorG.007	Mate-pair, Only Emulsion PCR	Noncleavable probe Sequence by ligation	Open source; cost effective
Single Molecule Sequencing	**Helicos BioSciences HeliScope**	Fragment, Mate-pair, Single molecule	Reversible Terminators	High multi-plexing ability, no template amplifi cation needed
	Pacific BioScience PacBio HRS	Fragment/ only Single molecule	Real Time	Longest reads, no template amplification needed, real time

Application of DNA-based authentication in medicinal plants

Several plants are being distinguished with the help of this new system of identification. Two species of the parasite *Cuscuta* (*C. reflexa* and *C. chinensis*) have been distinguished with primers OPC-1, OPC-02, OPC-03, OPC-04, OPC-05, OPC-06,

OPC-07 and OPC-08, (32, 33) while characterizing *Clitoria ternatea* at inter-zonal level identified complete monomorphism with primer OPN-02 which makes OPN-02 a good choice for the identification of this herb. Molecular characterization of *Convolvulus pluricaulis* revealed that primer OPN-09 is the species specific primer for the herb (8). A common band of 2.2 kb amplified by Primer OPN-05 was found when different accessions of *Evolvulus alsinoides* were studied through RAPD analysis (34). The roots of *Cissampelos pareira* var. *hirsuta* (Buch.-Ham. ex DC.) Forman) generally named as *Patha* in India is substituted with two other species, viz., *Cyclea peltata* (Lam.) Hook.f. & Thomson and *Stephania japonica* (Thunb.) Miers. ISSR profiles (35) distinguished genuine raw drug of *Patha* from its substitutes/adulterants to guarantee the quality and legitimacy of this drug in the market (8).

Usage of rbcL sequences alone to make assignments to species (or species groups) for 85% of all root samples examined, permitting a detailed examination of the ecological factors that contributed to the subterranean spatial organization of plant diversity in an old-field community (36). Likewise, DNA barcoding can provide identification where material has been processed in one way or another, such as analyzing the diet of herbivores (37, 38), food products (39), or the components of herbal medicines (40). matK DNA barcodes are also used to highlight misidentified plant species in herbal supplements (41). There are reports on the usage of DNA barcoding as an efficient tool for tracing medicinal plant and aromatic plants to provide a safer food supplement for consumers (42). DNA metabarcoding has been applied to assess the quality and validate herbal drugs in the industrial context (43). A novel nanoparticle- DNA barcoding hybrid system named NanoTracer has also been used which helps for hasty and molecular level invention of any food and herbal materials (44).

The development of bioinformatics provides a broad spectrum for combining unlinked and scattered genomic data of medicinal plants and facilitates the identification of plant sources and discovery of new drugs for future therapeutics (45). The development and application of toxicogenomics for finding the drug interaction and toxic chemicals in the herbs helped tracing adulterants in Chinese herbal medicines (46). There are reports on the use of NGS in phylogenetic analysis of two lineages of monocots, the Asparagales and the grasses, using Illumina data (80 – 120-bp reads) (47).

Emergence of herbal Genomics

The medicinal value of each plant is the output of the secondary metabolites produced in different pathways. These pathways need to be explored to know the potential bioactivities of the plants and also for the new drug discovery. Hence the whole nuclear and chloroplast genomes needed to be sequenced to understand these metabolic pathways. An attempt for the analysis of genome sequencing of various medicinal plants and their functional genomics is initiated by "Herb Genome Programme" (48). Genomic information, together with transcriptomic, proteomic and metabolomic data, can therefore be used to predict the secondary metabolic pathways of herbs (49). The genomes of some commonly used medicinal mushroom *Ganoderma lucidum* and herbs *Salvia miltiorrhiza* Bunge and *Catharanthus roseus* (L.) G. Don have already sequenced and they emerged as valuable models for studying the genetic and metabolic activities of herbs (50-52). The whole nuclear and chloroplast genomes of holy basil (*Ocimum sanctum* L.) have been sequenced and analyzed for the expression of various metabolite pathways which helps to relate the biosynthetic pathways of related species (53). The whole genome data of *Azadirachta indica* A. Juss., *Ziziphus jujuba* Mill., *Gelsemium sempervirens* (L.) J.St.-Hil. *Camptotheca acuminata* Decne. *Calotropis gigantea* (L.) Dryand. are also released recently to the herbal genomics (54-57). Molecular studies have been used in the identification of several other medicinal plants (Table 2).

Application in identification of polyherbal formulations

The traditional medicines and the herbal products have attracted global attention and the commercial interest augmented the encouragement for adulteration and substitution in the herbal market. This has prompted and challenging the herbal pharmacovigilance to build up novel techniques for the complete assessment and monitoring of herbal products. A study reported DNA metabarcoding to authenticate seventy-nine Ayurvedic herbal products sold as tablets, capsules, powders, and extracts were randomly purchased via e-commerce and pharmacies across Europe (186). The low level ingredient fidelity in their analysis raises concerns of fidelity and quality of herbal drugs and highlights the necessity for quality control of marketed herbal products and shows DNA metabarcoding as an effective analytical approach to authenticate complex polyherbal formulations. In an another study (180), RAPD technique was employed for determination of the components in an Ayurvedic herbal prescription, "*Rasayana Churna*" for the simultaneous identification and quantification of *Tinospora cordifolia, Emblica officinalis* and *Tribulus terestris* in *Rasayanachurna*. Primer OPC-6 clearly differentiates all components of *Rasayanachurna* (180). This has proved as an efficient, precise and sensitive method for identifying components for *Churnas* and will contribute significantly in quality control. There are reports that analyzed the Chinese herbal formulation "*Ruyi Jinhuang*" composed of nearly 10 ingredient drugs based on

high-throughput sequencing and DNA barcoding for authentication of ingredients and identification of adulterants and toxic compounds (187). Their findings established an effective approach for monitoring the biological composition of traditional Chinese medicines based on high-throughput sequencing and DNA barcoding. Real-time PCR was included to validate the accuracy of identification. This study demonstrates the application of high-throughput sequencing combined with real-time PCR to detect the biological and toxic ingredients in herbal preparations (187).

Challenges and constrains

Isolation of quality DNA is essential step in molecular characterization even though a particular molecular marker is selected. There are many issues reported for the isolation of DNA from plants. Isolated DNA showed colored substances, polysaccharides and phenolic compounds (188-89). The best method for the extraction and purification of DNA from a particular plant or drug sample needs to be established empirically. Techen and colleagues 2006, (190) showed that the success of PCR was dependent on both the type of source material (raw plants, herbal teas, tablets, capsules) as well as the specific brand of commercial DNA extraction kit used.

Sequence-based analyses sometimes fail to distinguish between species because of the significant similarity between their DNA sequences in the amplified region. RAPD primers are able to distinguish taxa below the species level (191), because RAPD analysis reflects both coding and non-coding regions of the genome (209). However, some of the problems with RAPD are related to reproducibility, designing appropriate primers and amplification of RAPD-PCR products. PCR conditions constitute one of the crucial factors for obtaining amplified products, especially for plants (192). Even though ISSR markers do have more value compared to RAPDs, the marker has the reproducibility issues and moreover it is a co dominant marker. It has been found that DNA barcoding fail to distinguish recently diverged species (193). In the case of SCAR technology it needs prior sequence data for designing primers. Primer designing in Loop Mediated Isothermal Amplification (LAMP) technology is complex; a minimum of two primer pairs is required to identify six different regions of target gene/DNA sequence (8).

Identification of polyherbal formulations is still a challenging task due to the difficulties associated with complex DNA isolation procedures. In addition to the degradation of DNA while processing or preparation of formulation adversely affects the sequencing. Due to the lack of strict regulatory controls, improper manufacturing process, this popular herbal product needs more scientific validation.

Even though a universal barcode has been established for plant species identification it is found to be not applicable for most of trees and herbs and which is exemplified while working on *Dendrobium* species (194). A case study with Indian Berberry species also confirmed the non-applicability of universal barcode when work with complex plant groups (195). The next generation technologies can circumvent all the quandaries of other molecular techniques.

Discussion

Medicinal plants represent the valuable source of traditional and modern medicine. The commercialization of raw drug mate rials has led to the increased use of adulterants and substitutes in the trade of medicinal plants. The gradual deterioration in the knowledge about the identification of medicinal plants has led to many misinterpretations and has put many herbal drugs in controversial position. The international trade of herbal products is one of the major forces in the global economy with increased demand in both developed and developing countries. In addition to ambiguity in nomenclature, the crude drugs sold in the market are adulterated or substituted by quite unrelated plant materials. Thus, authentication of botanical source of plant from which the raw drugs are obtained for research or medicinal use is a necessity to accomplish satisfactory results and also to sustain the efficacy and therapeutic property of the preparations in which these plants are used. We have tried to present a comprehensive review on strategies related to identification of medicinal plants used in Indian medicinal system based on the various DNA markers. DNA based authentication of medicinal plants can be useful as a tool for quality control and safety monitoring of herbal pharmaceuticals and neutraceuticals and will significantly add to the medical potential of herbal products (8).

The greatest drawbacks in support and promotion of herbal products are adulteration of their market samples. Due to this adulteration and altered efficacy, the faith in crude drug promotion has declined (196). One of the encumbrances in the approval of herbal formulations is the lack of standardization and quality control profiles. Owing to the complex nature and inherent variability of the chemical constituents of plant-based drugs, it is difficult to establish quality control parameters using phytochemical tools (197). The pitfalls associated with the common conventional pharmacognosy methods like macro-microscopic examination and phytochemical analysis have insisted researchers to the exploration of ultimate solutions for authentication of herbs and formulations (198). Expected chemicals in the herbal plant targeted

for medicinal use could vary with the genomic or environmental variability of the species (199).

DNA-based techniques have been widely used for authentication of plant species of medicinal importance. This is especially useful in case of those that are frequently substituted or adulterated with other species or varieties that are morphologically and/or phytochemically indistinguishable. DNA markers use nucleotide sequences to identify species; it takes preference over the other two markers being not age dependent, tissue specific and having a higher discriminating power. Therefore, characterization of plants with such markers is an ideal approach for identification of medicinal plant species and populations/varieties of the same species. DNA markers have superiority in identifying medicinal plants compared to other markers. According to one study on the various DNA markers, LAMP, SCAR and DNA barcoding are ideal for authentication (8). DNA markers are very reliable for informative polymorphisms and as the genetic composition are unique for each species and are not affected by age, any physiological conditions and as well as environmental conditions (200). British and Chinese pharmacopoeia has already started including modern DNA-based details to distinguish herbs (201- 203). It is high time to focus the molecular details of medicinal plants used in Indian systems of medicines for the proper identification. Indian research institutes on traditional systems coming under AYUSH also should implement these details in their pharmacopoeia. A reference library of DNA markers of traditional medicinal plants is surely required and should be established. The availability of certified taxonomic specimens in herbaria and raw drug samples in museums are indeed required for the unambiguous authentication through final visual assessment and analysis. Now the NGS technologies have proven its application in plant science context also, it is essential to emphasis on this advanced tool for the comprehensive genomic analysis and genetic conservation of Indian medicinal plants. NGS methods differ in read length, the types and prevalence of errors and the number of reads created per run; different approaches are needed to deal with the data in terms of quality control, assembly, and analysis. This presents a major challenge in terms of computational resources, innovation and application (20).

Conclusion

This review focused the need of application of novel strategies for authentication and identification of both raw drugs and final herbal products to ensure the quality, efficacy and fidelity of the herbal system. The pharmacopeias should include the new techniques of identification methods which are accompanied by the modern science and technology developments. Even though we are having these many molecular data of medicinal plants for authentication which is mainly focused on molecular code of leaves, the correct identification is possible only when the mentioned part of concerned drug is barcoded. The emergence of herbal genomics and NGS technologies will open a wide spectrum of global approval and consistency to these medicinal plants and their products.

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Competing Interests

The authors declare that they have no competing interests.

Authors' contribution

RM contributed to the intellectual content, conceptualization of the topic, designing, data acquisition and interpreting analysis for the manuscript, DKG contributed literature review and data tabulation, EB contributed in data compilation, SKKN contributed manuscript review and manuscript editing and revised to final format.

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