FINGERPRINTING TECHNIQUES IN HERBAL STANDARDIZATION

Kirti M. Kulkarni, Leena S. Patil, Mrs. Vineeta V. Khanvilkar, Dr. Vilasrao J. Kadam

1Department of Quality Assurance, Bharati Vidyapeeth’s College of Pharmacy, C.B.D. Belapur, Navi Mumbai, Maharashtra, India.

ARTICLE INFO

Article history
Received 18/02/2014
Available online 28/02/2014

Keywords
Herbal Formulations,
Standardization,
Fingerprinting,
Chromatographic Fingerprinting,
DNA Fingerprinting.

ABSTRACT

Medicinal plants have played a key role in world health. In spite of great advances observed in modern medicines; herbal formulations have reached extensive acceptability as therapeutic agents for treatment of several diseases. Herbal drug technology is used for converting botanical materials into medicines, where standardization and quality control with proper integration of modern scientific techniques and traditional knowledge is important. ‘Standardization’ is a process of evaluating the quality & purity of crude drug by means of various parameters like morphological, microscopical, physical, chemical & biological observation. However herbal medicines differ from that of the conventional drugs and some innovative methods are necessary for quality assessment of herbal drugs. Fingerprint analysis approach is the most potent tool for quality control of herbal medicines because of its accuracy and reliability. Fingerprinting is a process that determines the concentrations of a set of characteristic chemical substances in an herb. Based on the conception of phytoequivalence, the chromatographic and DNA fingerprints of herbal medicines could be utilized for addressing the problem of quality control of herbal medicines. This article will review advanced techniques of chromatographic and DNA fingerprinting in standardization of herbals.

Please cite this article in press as Kirti M. Kulkarni, et al. Fingerprinting Techniques in Herbal Standardization. Indo American Journal of Pharm Research.2014;4(02).

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INTRODUCTION
Herbal drugs have been used since ancient times as medicines for the treatment of range of diseases. Medicinal plants have played a key role in the world health. In recent decades, in spite of the great advances observed in modern medicine, plants still make an important contribution to health care[1-3]. This increased use of herbal medicines is due to several reasons namely, inefficiency of conventional medicines (e.g. side effects and ineffective therapy), abusive use of synthetic drugs resulting in side effects, large percentage of world’s population does not have access to conventional pharmacological treatment and folk medicines and ecological awareness which suggest that natural products are harmless. According to an estimate of the World Health Organization (WHO), about 80% of the world population still use herbs and other traditional medicines for their primary health care needs.

Of the 252 drugs considered as basic and essential by the WHO, 11% are exclusively of plant origin and a significant number of synthetic drugs are obtained from natural precursors. Examples of important drugs obtained from plants are digoxin from Digitalis spp., quinine and quinidine from Cinchona spp., vincristine and vinblastine from Catharanthus roseus, atropine from Atropa belladonna and morphine and codeine from Papaver somniferum[3]. According to WHO, herbal medicines include herbs, herbal materials, herbal preparations and finished herbal products.

Herbs include crude plant materials, such as leaves, flowers, fruits, seeds, stems, woods, barks, roots, rhizomes or other plant parts, which may be entire, fragmented or powdered.

Herbal materials in addition to herbs include; fresh juices, gums, fixed oils, essential oils, resins and dry powders of herbs. Various procedures, such as steaming, roasting or stir-baking with honey are carried out for their preparation.

Herbal preparations are finished herbal products and may include comminuted or powdered herbal materials, or extracts, tinctures and fatty oils of herbal materials. These are produced by extraction, fractionation, purification, concentration, or other physical or biological processes.

Finished herbal products consist of herbal preparations made from one or more herbs which may contain excipients in addition to the active ingredients[1].

STANDARDIZATION
As commercialization of the herbal medicine has happened, assurance of safety, quality and efficacy of medicinal plants and herbal products has become an important issue. Herbal raw materials are prone to a lot of variation due to several factors, the important ones being the identity of the plants and seasonal variation (which has a bearing on the time of collection), the ecotypic, genotypic and chemotypic variations, drying and storage conditions and the presence of xenobiotic[1].

Also one of the major problem faced by herbal drug industry is unavailability of rigid quality control profile for herbal materials and their formulations. As per American Herbal Product Association, “Standardization refers to the body of information and control necessary to obtain product material of reasonable consistency”[3]. ‘Standardization’ is a process of evaluating the quality and purity of herbal drug on the basis of various parameters like morphological, microscopic, physical, chemical & biological parameters.

CONVENTIONAL METHODS FOR STANDARDISATION OF HERBAL FORMULATION
Phytochemical standardization encompasses all the possible information generated with regard to the chemical constituents present in an herbal drug. Hence, the phytochemical evaluation for standardization purpose includes the following:

- Preliminary testing for the presence of different chemical groups. (e.g., total alkaloids, total phenolics, total triterpenic acids, total tannins etc.)
- Quantification of chemical groups of interest.
- Establishment of fingerprint profiles based upon single or multiple markers[1].

Standardization of herbal raw drugs include passport data of raw plant drugs, botanical authentication which include microscopic & molecular examination, physical parameters like moisture content, ash value, extractive value etc., identification of chemical composition by various chromatographic techniques and determination of biological activity of the whole plant.
Moisture content, Ash value, Extractive value, Viscosity, Density, Bitterness value, Solubility, Swelling index, Foaming index, Specific gravity,

Color, Odor, Taste, Texture and Fracture

Macroscopic

Moisture content, Ash value, Extractive value, Viscosity, Density, Bitterness value, Solubility, Swelling index, Foaming index, Specific gravity,

Qualitative, Quantitative, SEM studies, Powder studies

Microscopic

Physical

Botanical

Standardization Of Herbal Drugs

Biological

Microbial contamination, Pharmacological evaluation, Toxicological studies

Chemical

Chromatographic techniques, Heavy metal, Pesticide residue, Mycotoxins

Figure. 1: Methods for herbal standardization.
Standardization of herbal formulation requires implementation of Good Manufacturing Practices (GMP). In addition, study of various parameters such as pharmacodynamics, pharmacokinetics, dosage, stability, self-life, toxicity evaluation, chemical profiling of the herbal formulations is considered essential. Good Agricultural Practices (GAP) in herbal drug standardization are equally important.

Table 1: General testing parameters for characterization and standardization of herbal medicines

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**ADVANCED TECHNIQUES FOR HERBAL STANDARDIZATION**

Quality control of herbal medicines is a tedious and difficult job. Herbal medicines differ from that of the conventional drugs and so some innovative methods are necessary for quality assessment of herbal drugs. Fingerprint analysis approach is the most potent tool for quality control of herbal medicines because of its accuracy and reliability. Fingerprinting is a process that determines the concentrations of a set of characteristic chemical substances in an herb. Knowing the relative concentrations is a means of assuring the quality of herbal preparations. It can serve as a tool for identification, authentication and quality control of herbal drugs. Based on the conception of phytoequivalence, the chromatographic fingerprinting and DNA fingerprints of herbal medicines could be utilized for addressing the problem of quality control of herbal medicines.
Fingerprinting techniques in herbal standardization

**Fingerprinting Techniques**

**Chromatographic Fingerprinting**
1. Thin Layer Chromatography (TLC)
2. High Performance Thin Layer Chromatography (HPTLC)
3. High Performance Liquid Chromatography (HPLC)
4. Gas Chromatography (GC)
5. Hyphenated Techniques

**DNA Fingerprinting**

**Hybridization Based Methods**
1. Restriction Fragment Length Polymorphism (RFLP)
2. Variable Number Tandem Repeats (VNTR)

**PCR Based Method**
1. Randomly Amplified Polymorphic DNA (RAPD)
2. Arbitrarily Primed Polymerase Chain Reaction (AP-PCR)
3. Amplified Fragment Length Polymorphism (AFLP)
4. Simple Sequence Repeats (SSR)

**Sequence Based Methods**
1. Single nucleotide polymorphism (SNP)
2. Simple tandem repeats (STR)

Figure 2: Fingerprinting techniques in herbal standardization
Chromatographic fingerprinting:

Chromatographic fingerprinting is the most powerful approach for the quality control of herbal medicines. Chromatographic fingerprint of Herbal Medicine is a chromatographic pattern produced from extract of some common chemical components which may be pharmacologically active or have some chemical characteristics\(^1\). This chromatographic profile should be featured by the fundamental attributions of “integrity” and “fuzziness” or “sameness” and “differences” so as to chemically represent the herbal medicines investigated. This suggest that chromatographic fingerprint can successfully demonstrate both “sameness” and “differences” between various samples and the authentication and identification of herbal medicines can be accurately conducted even if the number and/or concentration of chemically characteristic constituents are not very similar in different samples of herbal medicine. Thus chromatographic fingerprint should be considered to evaluate the quality of herbal medicines globally; considering multiple constituents present in the herbal medicines\(^1,5-11\).

This technique can be employed for identification and authentication as well as for determination of various adulterants and contaminants and for standardization purpose. In contrast to macroscopic, microscopic and other molecular biological methods this technique is not restricted to raw herbs, but can also be applied to pharmaceutical preparations. Chromatographic fingerprinting can be carried out using techniques such as Thin layer chromatography (TLC), High performance thin layer chromatography (HPTLC), High performance liquid chromatography (HPLC), Gas chromatography (GC) and other hyphenated techniques.

Thin Layer Chromatography (TLC)

It is one of the most popular and simple chromatographic technique used of separation of compounds. In the phytochemical evaluation of herbal drugs, TLC is being employed extensively for the following reasons:

- It enables rapid analysis of herbal extracts with minimum sample clean-up requirement.
- It provides qualitative and semi-quantitative information of the resolved compounds.

In TLC fingerprinting, the data that can be recorded using a high-performance TLC (HPTLC) scanner which includes information like chromatogram, retardation factor (Rf) values, the color of the separated bands, their absorption spectra, \(\lambda\) max and shoulder inflection/s of all the resolved bands. All of these, together with the profiles on derivatization with different reagents, represent the TLC fingerprint profile of the sample. The information so generated has a potential application in the identification of an authentic drug, in excluding the adulterants and in maintaining the quality and consistency of the drug\(^1,5\).

Eg: TLC fingerprinting was done on the methanolic extract of Sitopaladi churna for determination of piperine using Silica Gel G plate and Toluene: Ethyl acetate: Formic acid (5:3.5:0.5 v/v/v) as mobile phase. Retention factor of piperine was found to be 0.69 (shown by peak 7) at 342 nm\(^{12}\).

![Figure. 3: TLC of Sitopaladi churna](image)

High Performance Thin Layer Chromatography (HPTLC)

HPTLC is the common fingerprinting method mainly used to analyze the compounds with low or moderate polarities. HPTLC technique is widely used in the pharmaceutical industry for quality control of herbs and health products, identification and detection of adulterants, substituents in the herbal products and also helps in the identification of pesticide contents and Mycotoxins\(^{1,13}\).

HPTLC method has several advantages which are as follows:

- Several samples can be run simultaneously by use of a smaller quantity of mobile phase as compared to HPLC.
- Mobile phases of pH 8 and above can be used for HPTLC.
- Repeated detection (scanning) of the chromatogram with the same or different conditions.
- HPTLC has been investigated for simultaneous assay of several components in a multi-component formulation. With this technique, authentication of various species of plant as well as the evaluation of stability and consistency of their preparations is possible.
Eg: HPTLC technique was reported for simultaneous determination of beta-sitosterol-d glucoside and withaferin A in *Ashwagandha* formulations using silica gel GF60 as stationary phase and Chloroform: Methanol (8:2) as mobile phase. Retention factor was found to be 0.21 (peak 1) and 0.59 (peak 2), respectively\(^\text{[14]}\).

![HPTLC of Ashwagandha formulation](image1.png)

**Figure 4. : HPTLC of Ashwagandha formulation**

**High Performance Liquid Chromatography (HPLC)**

The preparative and analytical HPLC has been widely used for analysis of herbal medicines because of its high separation capacity. It can be employed to analyze almost all constituents of herbal products provided that an optimized procedure is developed which involves optimization of mobile phase and stationary phase along with other chromatographic parameters. There are basically two type of Preparative HPLC Low pressure HPLC (typically under 5 bar) and High pressure HPLC (pressure > 20 bar)\(^\text{[1,5,7,13]}\).

Reversed phase columns are the most popular columns used in the analytical separation of herbal medicines. In order to obtain better separation, some new techniques are developed in the research field of liquid chromatography like Micellar electrokinetic capillary chromatography (MECC), High speed counter current chromatography (HSCCC), Low pressure size exclusion chromatography (SEC), Reversed phase ion-pairing HPLC (RPIPC-HPLC) and strong anion exchange HPLC (SAX-HPLC).

Eg: Kankasava is a polyherbal formulation prepared with Kanaka and other ingredients which is used in chronic bronchitis, asthmatic cough and breathlessness. A simple, precise, accurate RP- HPLC method was developed for the quantitative estimation of atropine using column RP C-18 (250mmx4.6mmx5 micron) and mobile phase which is mixture of methanol and 10 mM dihydrogen phosphate buffer in a ratio of 50:50 v/v at a flow rate of 1 ml/min, and analysis was screened with UV detector at 254 nm. The retention time for standard atropine sulphate was found to be 4.0667 minutes\(^\text{[15]}\).

![HPLC chromatogram for atropine sulphate](image2.png)

**Figure 5. : HPLC chromatogram for atropine sulphate (Rt =4.0667 min)**
Gas Chromatography (GC)

As some of the bioactive constituents of herbal medicines are volatile, GC analysis can often be used for authentication and quality control. The high selectivity of capillary columns enables separation of many volatile compounds simultaneously within comparatively short times. However, the most serious disadvantage of GC is that this method is not convenient for the analysis of samples which are thermolabile and non-volatile.\(^{[1,7,13]}\)

Eg: The identification and quantification of chemical constituents present in polyherbal oil formulation (Megni) was done by GC for determination of Eugenol using DB-5 fused silica capillary column and helium as a carrier gas. The retention time was found to be 8.63 min\(^{[16]}\).

![Figure. 6: GC chromatogram of Eugenol (Rt = 8.63 min)](image)

Hyphenated Techniques

Chromatographic separation techniques can be coupled to various detection techniques such as mass spectrometry (MS), nuclear magnetic resonance (NMR), infrared spectroscopy (IR) etc. These hyphenated techniques provide information about the structure of the compound present in chromatogram and thus provide higher sensitivity in comparison to conventional approaches. Various hyphenated techniques used include:

- Liquid chromatography- mass spectrometry (LC-MS)
- Liquid chromatography – nuclear magnetic resonance (LC-NMR)
- Gas Chromatography-Mass Spectroscopy (GC-MS)
- Gas Chromatography Fourier Transform Infrared spectrometry (GC-FTIR)

Liquid chromatography- mass spectrometry (LC-MS)

Importance of this technique has strongly increased because this technique can be used to characterize wide variety of plant constituents ranging from small molecules to macromolecules such as peptides, proteins, carbohydrates and nucleic acids. Recent advances includes electrospray, thermospray, and ionspray ionization techniques which offer unique advantages of high detection, sensitivity and specificity. Isotopes pattern can be detected by this technique\(^{[1,4,5,9]}\).

Liquid chromatography – nuclear magnetic resonance (LC-NMR)

The combination of chromatographic separation technique with NMR spectroscopy is one of the most powerful and time saving method for the separation and structural elucidation of unknown compound and mixtures, especially for the structural elucidation of light and oxygen sensitive substances. The online LC-NMR technique allows the continuous registration of time changes as they appear in the chromatographic run automated data acquisition and processing in LC-NMR improves speed and sensitivity of detection. The recent introduction of pulsed field gradient technique in high resolution NMR as well as three-dimensional technique improves application in structure elucidation and molecular weight information. These new hyphenated techniques are useful in the areas of pharmacokinetics, toxicity studies, drug metabolism and drug discovery process\(^{[1,4,6,9]}\).

Gas-chromatography- mass spectrometry (GC- MS)

Gas chromatography equipment can be directly interfaced with rapid scan mass spectrometer of various types. GC-MS is unanimously accepted methods for the analysis of volatile constituents of herbal medicines, due to their sensitivity, stability and high efficiency. Especially, the hyphenation with MS provides reliable information for the qualitative analysis of the complex constituents. The flow rate from capillary column is generally low enough so that the column output can be fed directly into ionization chamber of MS\(^{[1,4,6,9]}\).
The simplest mass detector in GC is the Ion Trap Detector (ITD). In this instrument, ions are created from the eluted sample by electron impact or chemical ionization and stored in a radio frequency field; the trapped ions are then ejected from the storage area to an electron multiplier detector. The ejection is controlled so that scanning on the basis of mass-to-charge ratio is possible. The ion trap detector is remarkably compact and less expensive than quadrupole instruments. GC-MS instruments have been used for identification of hundreds of components that are present in natural and biological system.

Gas Chromatography Fourier Transform Infrared spectrometry (GC-FTIR)

Coupling capillary column gas chromatographs with Fourier Transform Infrared Spectrometer provides a potent means for separating and identifying the components of different mixtures.

Summary

Despite of widespread acceptance of chromatographic fingerprint techniques for quality control, the establishment of a characteristic fingerprint chromatogram for the quality control of herbal medicines remains a critical task. The ability to obtain a good chromatographic fingerprint depends on several factors such as extraction method, measurement instrument and measurement conditions (selection of mobile phase and stationary phase). However in contrast to microscopic, macroscopic and many molecular biology methods, chromatographic methods are not only restricted to raw herbs, but can also be applied to herbal preparations.

DNA fingerprint:

DNA analysis has been proved as an important tool in herbal drug standardization which is useful for the identification of phytochemically indistinguishable genuine drug from substituted or adulterated drug. DNA fingerprint genome remain the same irrespective of the plant part used while the phytochemical constituents will vary with the part of plant used, physiology and environment.

The other useful application of DNA fingerprinting is the availability of intact genomic DNA specificity in commercial herbal drugs which helps in distinguishing adulterants even in processed samples.

Types of DNA fingerprinting techniques used in plant genome analysis:

Various types of DNA-based molecular techniques are utilized to evaluate DNA polymorphism which include hybridization-based methods, polymerase chain reaction (PCR)-based methods and sequencing-based methods.

Hybridization-based methods:

Hybridization based methods use cloned DNA elements or synthetic oligonucleotides as probes to hybridize the DNA of interest. The probes are labeled with radioisotopes or with conjugated enzymes which catalyze a color reaction to detect hybridization. Hybridization based methods include following steps.

Figure. 7: Hybridization based method

DNA is first extracted from plant cell by various methods of extraction such as Cetyl triethyl ammonium bromide (CTAB) method, Phenol/ Chloroform extraction method, DNA trap method etc. DNA is then cleaved with restriction enzyme after which it is subjected to gel electrophoresis. As DNA molecules have net negative charge at neutral PH, these molecules migrate towards the positive terminal when placed in an electric field in a process known as electrophoresis which is performed in an agarose or polyacrylamide gel. Nucleic acids are loaded into slots in the gel and allowed to migrate towards the positive terminal. The pores in the gel act to sieve the molecules. So that mobility of a particular nucleic acid species depends on its length. All the molecules of a particular size move at approximately the same rate through the gel, forming a band, which gradually increases in width during electrophoresis because of diffusion. After gel electrophoresis, DNA sequence is determined by Southern blotting technique. DNA molecules separated by gel electrophoresis are transferred to nitrocellulose or nylon membrane.
The DNA is denatured either prior to or during transfer by placing the gel in an alkaline solution, after which it is immobilized on the membrane by drying or UV induced cross linking to the filter. A radioactive DNA known as probe which are of two types (Tandem repeats which occurs as clusters among chromosomes and Dispersed repeats which are scattered all over chromosomes) containing the sequence of interest are then hybridized or annealed with the immobilized DNA on the membrane. The probe will anneal to form a double helix only with complementary DNA molecule on the membrane. Nonannealed probe is then washed off the membrane, and the washed membrane is exposed to X-ray film that detects the presence of the radioactivity in the bound probe, after which autoradiogram is developed where the dark bands show the positions of DNA sequences that has hybridized with the probe[19-20].

Hybridization-based methods include
a) Restriction Fragment Length Polymorphism (RFLP)
b) Variable Number Tandem Repeats (VNTR)

Restriction Fragment Length Polymorphism (RFLP)
In this technique, plants may be differentiated by analysis of patterns derived from cleavage of their DNA. Restriction polymorphism is detected by using a hybridization probe. RFLPs involves fragmenting a sample of DNA by a restriction enzyme, which can recognize and cut DNA wherever a specific short sequence (4-6 base pair recognition site) occurs, in a process known as a restriction digest. The resulting DNA fragments are then separated by length through a process known as agarose gel electrophoresis, and transferred to a membrane via the Southern blot procedure. PCR amplification of DNA is not required for this method. Hybridization of the membrane to a labeled DNA probe then determines the length of the fragments which are complementary to the probe[7,13,18,21].

Figure. 8 : Steps involved in restriction fragment length polymorphism

Limitations:
- Low sensitivity.
- Requirement of large amount of high quality genomic DNA which is difficult to obtain from processed herbs.
- Stability and reproducibility are low because the quality of the assay highly depends on DNA quality and technical factors[7].

Variable Number Tandem Repeats (VNTR)
This technique is similar to RFLP only the probe used for souther blotting exists as tandem repeats which occur as cluster among chromosomes. They show variations in length between individuals and each variant acts as an inherited allele[22].

Polymerase chain reaction based methods:
PCR enzymatically multiplies particular DNA sequence or loci of a template DNA with the help of arbitrary or specific oligonucleotide primers. PCR based procedures are difficult to standardize due to use of different DNA polymerase, different buffer formulations and different equipments used[7]. PCR based method involve following steps: Primers, original DNA (extracted from the plant cell) which is to be amplified, a specific type of DNA polymerase, and the necessary chemicals for DNA synthesis are mixed. Then following steps are carried out.
Figure. 9 : Steps involved in polymerase chain reaction

- **Denaturation**: DNA fragments are heated at high temperature temperature (95°C for 30 seconds or 97°C for 15 seconds) which reduces DNA double helix to single helix to single strand which become accessible to primer.

- **Annealing**: Here the temperature is lowered until the primers can hybridize, or bind to complementary regions on the DNA.

- **Extension**: The primers are used by DNA polymerase to initiate synthesis and new complementary strand of DNA are made.

The enzyme read opposing strand sequence and extends the primer by adding nucleotide in order in which they can pair

Once this synthesis is finished, the steps are repeated i.e. denaturation, renaturation, and synthesis. However problems can occur when the plant material of interest contains compounds such as phenolics which can interact with DNA and directly inhibit DNA polymerase or damage the structural integrity of DNA\(^{20,23}\).

Various PCR based techniques include:

a) Randomly Amplified Polymorphic DNA (RAPD)
b) Arbitrarily Primed Polymerase Chain Reaction (AP-PCR)
c) Amplified Fragment Length Polymorphism (AFLP)
d) Simple Sequence Repeats (SSR)

Randomly Amplified Polymorphic DNA (RAPD) and Arbitrarily Primed Polymerase Chain Reaction (AP-PCR)

These methods are most commonly used for primary assay which helps in screening the differences in DNA sequence of two species of plants.

In this method single arbitrarily chosen oligonucleotide is used as both the forward and reverse primer in PCR reaction. This sequence consists of about 10 nucleotide in case of RAPD and about 20 nucleotides in case of AP-PCR. Product is produced when the primer binds on opposite strands, in the reverse orientation and within an amplifiable distance. PCR fragments are generated from different locations of the genome, because there are multiple sites within the genome for the primer to bind. Thus, multiple loci may be examined simultaneously. Use of series of different primers, shows the generation of genetic fingerprint.

Figure. 10: Steps involved in RAPD

**Advantages**\(^{17}\)
Advantages
- Higher reproducibility, resolution, and sensitivity.
- Reliable and robust technology.
- Capability to amplify between 50 and 100 fragments at one time.
- No prior sequence information is needed for amplification.

Disadvantages
- It is expensive and time consuming due to digestion, ligation and two rounds of PCR.

Simple Sequence Repeats (SSR)
For this technique, prior sequence information is required. SSR markers or microsatellites also termed simple sequence length polymorphism (SSLP) or sequence tagged microsatellites (STMS) are tandem repeats scattered throughout the genome. They can be amplified using primers that flank these regions. The technique has been successfully used to construct detailed genetic maps of several plant species and to study genetic variation within populations of the same species. As the markers are usually species specific, their development is rather costly, but once they have been developed the method becomes quite inexpensive.7,22

Advantages
- Less amount of DNA is required than RFLP
- Assays are more robust

Disadvantages
Separate SSR primers are required for each species.
Sequence based methods:
Concerning this technique, DNA sequences from the nuclear and chloroplast genomes are used for identification of plants at several taxonomic level. Certain sequences such as the 5s rDNA spacer and internal transcribed spacer (ITS) regions between the 16S and 26S rDNA are highly variable and can thus be used for authentication at species level. Sequences such as nuclear 18S or plastidal rbcL, ndhF or matK however, are conserved amongst species and therefore suitable for discrimination at genus or family level. DNA sequenced based techniques have widely been used for authentication of herbs.

Various sequence based methods include:
  a. Single nucleotide polymorphism (SNP)
  b. Short tandem repeats (STR)

Single nucleotide polymorphism (SNP)
SNP is a DNA sequence variation occurring when a single nucleotide — A, T, C or G — in the genome (or other shared sequence) differs between members of a species[18].

Short tandem repeats (STR)
STR is a class of polymorphisms that occurs when a pattern of two or more nucleotides are repeated and the repeated sequences are directly adjacent to each other. The pattern can range in length from 2 to 10 base pairs (for example (CATG)n in a genomic region) and is typically in the non-coding intron region, making it junk DNA. By examining enough STR loci and counting how many repeats of a specific STR sequence, there are at a given locus, it is possible to create a unique genetic profile of an individual[18].

Advantages and disadvantages of DNA fingerprinting
Advantages[7]:
  o DNA markers are reliable for informative polymorphism as the genetic composition is unique for each species and is not affected by age, physiological conditions and environmental factors.
  o Low amount of material is required.
  o The techniques are independent from the physical form of the material.
  o DNA-based markers represent the most basic signature of an organism and are therefore much less affected by environmental influences than phenotypic markers.

Disadvantages[7]:
  o Expensive.
  o Some of the methods strongly depends on DNA quality which might be a problem in case of dried or processed materials.
  o Certain plant compounds or fungal contamination may also influence DNA extraction or PCR reaction.

CONCLUSION
The determination of common peaks in a set of chromatographic fingerprints provides useful qualitative and quantitative information on the characteristic components of herbal medicines investigated. Thus chromatographic fingerprint analysis serves as a promising quality control tool for herbal medicines[8].

DNA fingerprinting is another technique which is a promising tool for the authentication of medicinal plant species and for ensuring better quality herbs and nutraceuticals. DNA fingerprinting, apart from identifying alterations in the genotypes of plant species, can also used for the betterment of drug-yield by tissue culturing. DNA of interest can be stored as germplasm, which is then used for future cultivation as well as for the conservation of endangered plant species. Thus problem of quality assurance of herbal medicines has been solved to a great extent with the help of chromatographic and DNA fingerprint analysis.

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