



SHORT REVIEW ON DNA-FINGERPRINTING AND STANDARDIZATION OF HERBAL DRUGS

Sakshi Minocha

LLOYD School of Pharmacy, Greater Noida, U.P, India

ABSTRACT:

Medicinal plants play a key role in world health. In spite of new advances developed in modern medicines, herbal formulations are extensively used as therapeutic agents for treatment of several diseases. Herbal drug technology is used for converting botanicals materials into medicines. Standardization is a process of evaluating the quality & purity of crude drug by various parameters like morphological, microscopical, physical, chemical & biological observation. Fingerprint analysis approach is the most important in quality control of herbal medicines because of its accuracy and reliability.

DNA fingerprinting is defined as the application of genetic/ molecular marker techniques which is used to identify cultivars. Fingerprinting is a process as it determines the concentrations of chemical substances in an herb by several methods like RFLP, AFLP, RAPD, SSR sequencing of rDNA-ITS region and DNA bar-coding. Development of fingerprints based on DNA markers is necessary for proper identification and standardization of plant species.

INTRODUCTION:

The herbal raw material has many variations due to several factors like the identity of the plants and seasonal variation, the ecotypic, genotypic and chemotypic variations, drying and storage conditions and the presence of xenobiotic.

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. Herbal medicines use has increased due to several reasons like inefficiency of conventional medicines, abusive use of synthetic drugs resulting in side effects, large percentage of world's population does not have access to conventional pharmacological treatment and medicines. According to an estimate of the World Health Organization (WHO), about 80% of the world population still uses herbs and other

traditional medicines for their primary health care needs.

Herbal medicinal plant material is adulterated either accidentally or intentionally with herbs with similar related species which are morphologically similar or by materials from unrelated plants. Proper authentication process is necessary to prevent the adulteration of herbal plants with other plant materials. For the standardization of botanical preparations most of the regulatory authorities and pharmacopeias suggest macroscopic, microscopic and chemical evaluation.

STANDARDIZATION:

Standardization refers to the information and control which is important for obtaining product material of reasonable consistency. Standardization is a process which evaluates the

quality and purity of herbal drug on the basis of various parameters like morphological, microscopical, physical, chemical & biological parameters.

Out of these, the phytochemical profile is of special significance since it has a direct effect on

the activity of the herbal drugs. The fingerprint profiles serve as guideline to the phytochemical profile of the drug in ensuring the quality, while quantification of the marker serve as an additional parameter in assessing the quality of the sample.

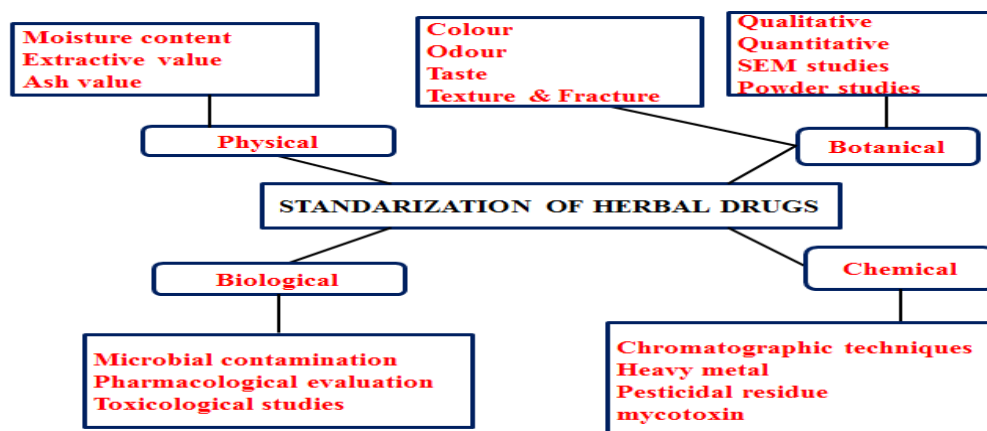


FIG: 1

CONVENTIONAL METHODS FOR STANDARDISATION OF HERBAL FORMULATION:

Phytochemical standardization consists of all the possible information regarding the chemical constituents present in herbal drug. Hence, the phytochemical evaluation for standardization 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.

Standardization of herbal raw drugs include 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.

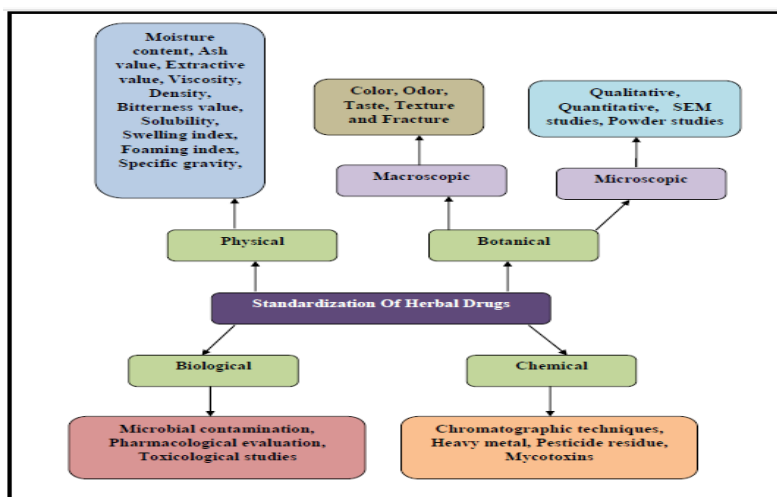


FIG: 2

WHO GUIDELINES FOR QUALITY STANDARDIZED HERBAL FORMULATIONS:

- 1) Quality control of crude drugs material, plant preparations and finished products.
- 2) Stability assessment and shelf life
- 3) Safety assessment; documentation of safety based on experience or toxicological studies.

4) Assessment of efficacy by ethno- medical information and biological activity evaluations.

The bioactive extract should be standardized on the basis of active principles or major compounds along with the chromatographic fingerprints (TLC, HPTLC, HPLC, and GC)

Parameters for standardization and Quality Control of herbal drugs

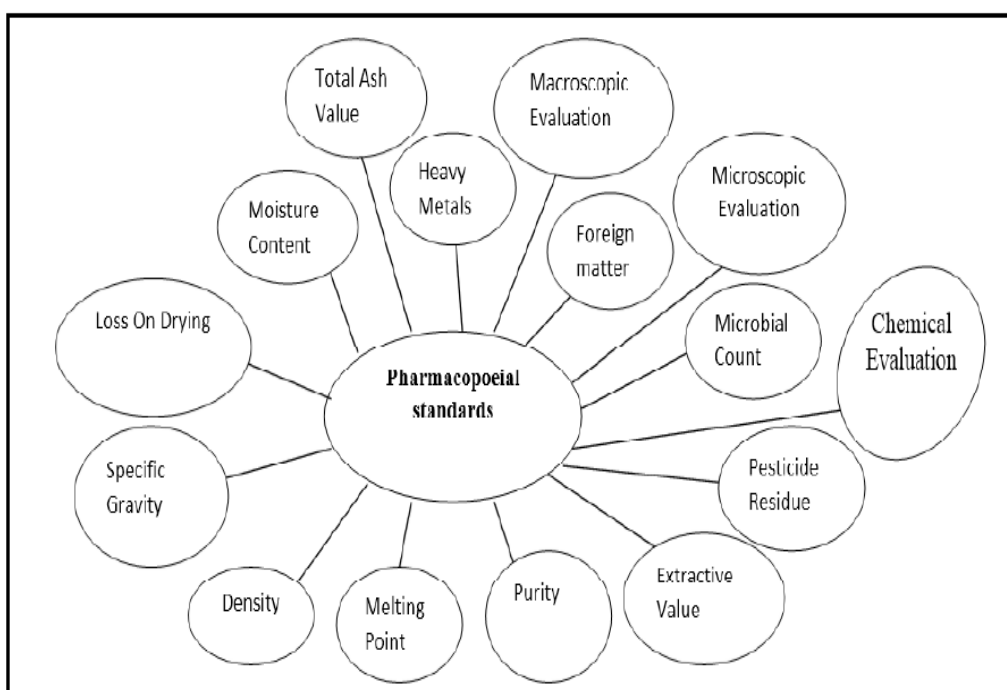


FIG: 3

Physical Evaluation- Contains botanical, macroscopic and microscopic descriptions which provide visual documentation of accurately identified material. A microscopic analysis assures the identity of the material and as an initial screening test for impurities.

Chemical Evaluation- Chemical analysis of the drug is done to assess the potency of herbal material in terms of its active principles. It covers screening, isolation, identification, and purification of the chemical components. It helps in determining the identity of the drug substance and possible adulteration.

Biological Evaluation- Pharmacological activity of certain drugs has been applied to evaluate and standardize them. The assays on living animals and on their intact or isolated organs can indicate the strength of the drug or their preparations.

Analytical Methods- It helps in determining identity, quality and relative potency.

ADVANCED TECHNIQUES FOR HERBAL STANDARDIZATION

Herbal medicines differ from conventional drugs; therefore some innovative methods are necessary for quality assessment of herbal drugs. Fingerprint analysis approach is one of the most potent parameter 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 phytoequivalence, the chromatographic fingerprinting and DNA fingerprints of herbal medicines can be used for treating the problem of quality control of herbal medicine.

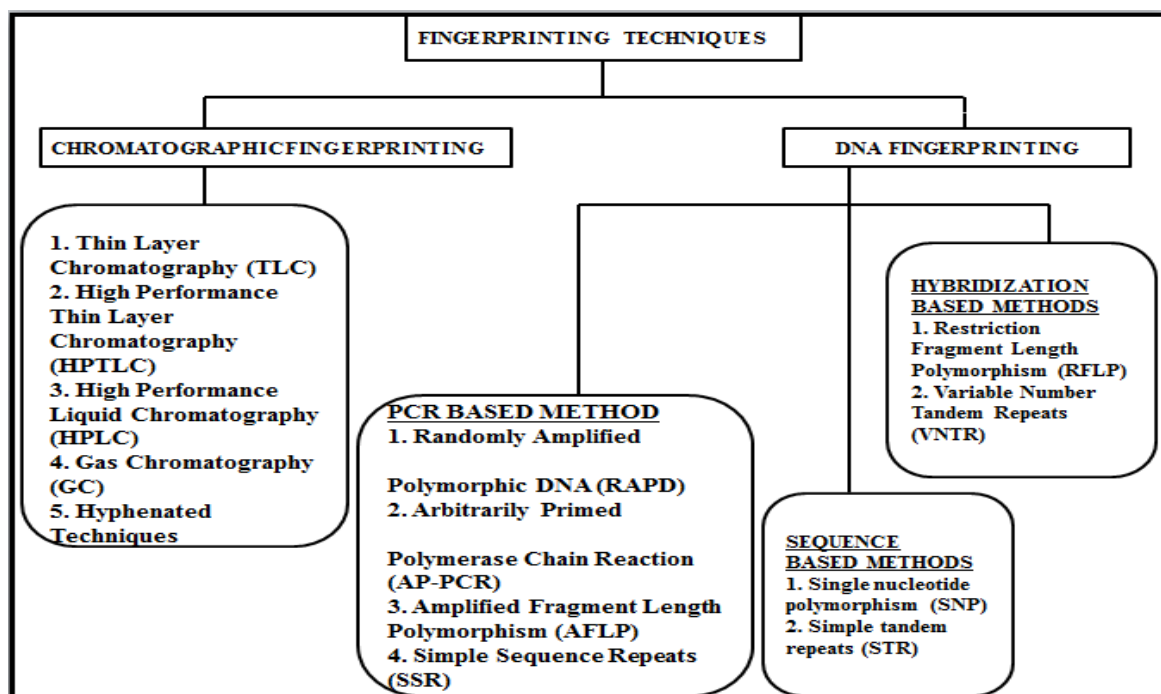


FIG: 4

DNA FINGERPRINTING:

DNA fingerprints are a bar-code like patterns generated by amplification of chromosomal DNA of an individual which can distinguish the uniqueness of this individual from another.¹¹ Also called DNA typing, genetic fingerprinting, DNA profiling and DNA typing.¹² DNA fingerprinting in plants can be applied to a number of applications and uses.¹³ DNA based marker analysis has been proven as an important tool in herbal drug standardization. DNA is the basic component of all living cells. The characteristics, traits and morphological features of plants are determined by the specific arrangement of DNA base pair sequences in their cell. DNA in cell is made of nucleotides i.e., Adenine, Guanine, Thymine and Cytosine and pentose sugar joined by phosphate bonds. These regulate the production of specific metabolites like enzymes and proteins.¹⁴ DNA fingerprinting is based on the identity of an organism at molecular level i.e., genetic characteristics. The technique of DNA Fingerprinting was discovered by Great Britain geneticist Alec J. Jeffrey in 1984. DNA profiling is primarily used in botanicals for protection of biodiversity, identifying markers for traits, identification of gene diversity and variation etc.

DNA/GENETIC MARKER:

It is a gene or DNA sequence with a known location on a chromosome and associated with a particular gene or trait. It can be described as a variation, which may arise due to mutation or alteration in the genomic loci that can be observed. A genetic marker may be a short DNA sequence, such as a sequence surrounding a single base-pair change (single nucleotide polymorphism SNP).

Some commonly used types of genetic markers are:

- RFLP (or Restriction fragment length polymorphism)
- AFLP (or Amplified fragment length polymorphism)
- RAPD (or Random amplification of polymorphic DNA)
- VNTR (or Variable number tandem repeat)
- Micro satellite polymorphism
- SNP (or Single nucleotide polymorphism)
- STR (or Short tandem repeat)
- SFP (or Single feature polymorphism)

Methodology of DNA fingerprinting:

The basic methodology of DNA profiling in plants involve first the isolation of DNA from

plant cells, quantification and quality assessment of isolation.

The further steps are of two types,

- 1) PCR based. (RAPD, ISSR, SSR)
- 2) Non PCR based. (RFLP)

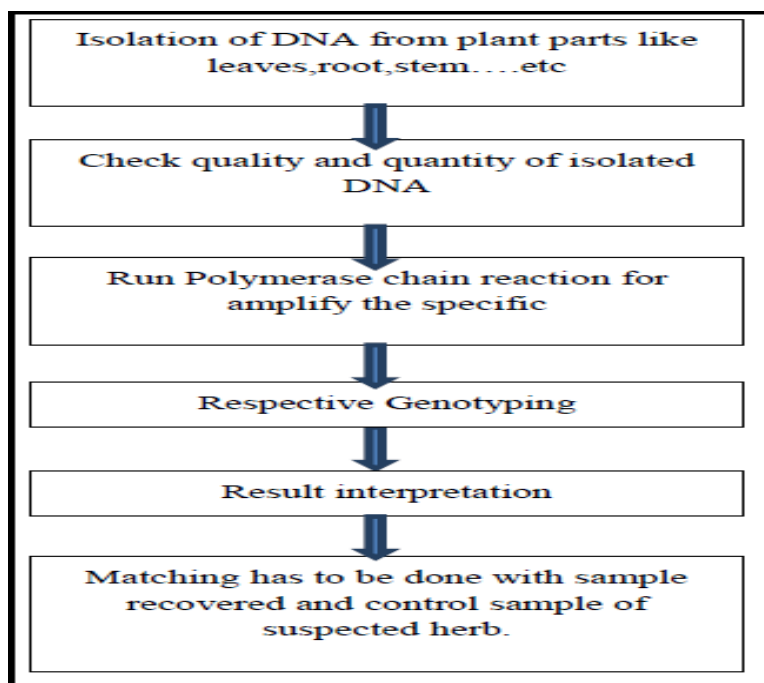


FIG: 5

DNA Isolation: DNA from plant tissue is isolated by removal of cell wall and nuclear membrane around the DNA and the separation of DNA from other cell components such as cell debris, proteins, lipids or RNA without affecting the integrity of the DNA. The most commonly preferred method is CTAB method. The DNA is isolated from tissues of plants, generally fresh leaves are preferred.

DNA Quantification and Quality assessment: DNA quantification and quality assessment is done by using UV-VIS spectrophotometry. Normally quality check is performed through the A260/A280 ratio that is 1.8 values shows the highest purity, if more than 1.8 shows the presence of RNA contamination and less than that indicates protein contamination.

Polymerase Chain Reaction (PCR) The DNA amplification by thermal cycling called Polymerase Chain Reaction. It is in vitro method that is used to amplify a specific DNA segment from small amounts of DNA template or duplex into millions of copies. Steps involved in PCR are:

- Heat Denaturation
- Annealing
- Primer Extension

Then Application of different genotyping methods like RAPD, AFLP, RFLP, ISSR are done.

Simple Sequence Repeats (SSR): SSR markers or microsatellites also termed simple sequence length polymorphism (SSLP) or sequence tagged microsatellites (STMS). Microsatellites are simple sequence repeats (SSRs), 1 to 6 nucleotides in length, which show a high degree of polymorphism. 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. SSRs can be detected by specific dyes or by radio-labeling using gel electrophoresis.

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.

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.

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.

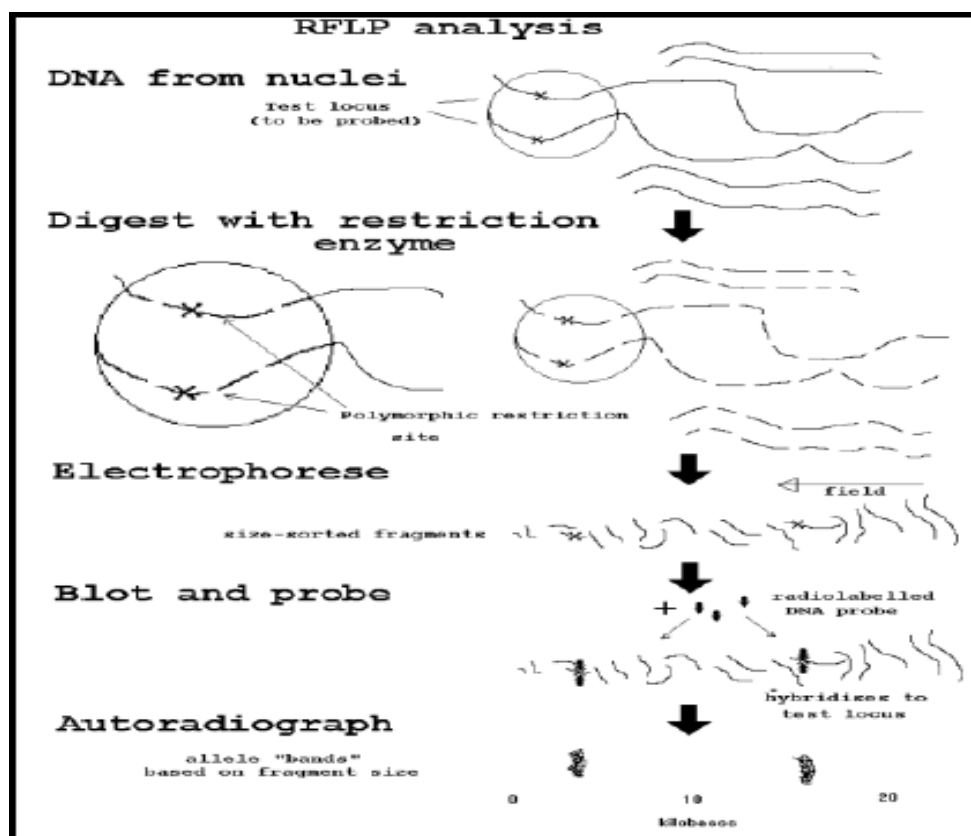


FIG: 6

Variable Number Tandem Repeats (VNTR):

It is a location in a genome where a short nucleotide sequence is organized as a tandem repeat. These can be found on many chromosomes, and show variations in length between individuals. Each variant acts as an inherited allele, and used for personal or parental identification. Their analysis is useful in genetics

and biology research, forensics, and DNA fingerprinting.

Randomly Amplified Polymorphic DNA (RAPD):

RAPD is one of the most commonly used primary assays for screening the differences in DNA sequences of two species of plants. RAPD consists of searching for the sequence using

random amplification. Plant genomic DNA is cut and amplified using short single primers at low annealing temperatures, and results in amplification at multiple loci. It is possible to determine the change in sequence pattern by running a 2-dimensional electrophoresis gel. As the band is identified, the gel is cut, and the DNA is isolated and sequenced.

Amplified Fragment Length Polymorphism (AFLP):

AFLP is a PCR-based derivative method of RFLP in which sequences are selectively amplified using primers. AFLP uses restriction enzymes to cut genomic DNA, followed by ligation of adaptors to the sticky ends of the restriction fragments. The amplified and labeled restriction fragments are separated on denaturing gels or by capillary electrophoresis. The complexity of the AFLP profile depends on the primers and restriction enzymes chosen and on the level of sequence polymorphism between the tested DNA samples.

Marker	Advantages	Disadvantages
RFLP	<ul style="list-style-type: none"> • Unlimited number of loci • Can be converted to SCARs • Robust in usage • Detects in related genomes • No sequence information required 	<ul style="list-style-type: none"> • Fairly expensive • Large quantity of DNA needed • Often very low levels of polymorphism • Needs considerable degree of skill
RAPD	<ul style="list-style-type: none"> • Results obtained quickly & Fairly cheap • No sequence information required • Relatively small DNA quantities required • High genomic abundance 	<ul style="list-style-type: none"> • Highly sensitive to laboratory changes • Low reproducibility within and between laboratories • Cannot be used across • Populations nor across species
ISSR	<ul style="list-style-type: none"> • Highly polymorphic • Robust in usage 	<ul style="list-style-type: none"> • Species-specific
SSR	<ul style="list-style-type: none"> • Fast and Robust • Highly polymorphic • Can be automated • Only very small DNA 	<ul style="list-style-type: none"> • High developmental and Start up costs • Species-specific • Sometimes difficult interpretation because of stuttering
AFLP	<ul style="list-style-type: none"> • Small DNA quantities required • No sequence information required • Can be automated • Can be adapted for different uses 	<ul style="list-style-type: none"> • Marker clustering • Can be technically challenging • Evaluation of up to 100 loci

Chromatographic Fingerprinting:

Chromatographic fingerprinting is the approach useful for the quality control of herbal medicines. Chromatographic fingerprint of an Herbal Medicine is a chromatographic pattern of the extract of chemical components of pharmacologically active and or chemical

characteristics. With the help of chromatographic fingerprints, the identification of herbal medicines can be accurately done.

Thin Layer Chromatography (TLC): Thin layer chromatography is simply known as TLC. It is one of the most popular and simple chromatographic technique used of separation of

compounds. 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 includes the chromatogram, retardation factor (Rf) values, the color of the separated bands, their absorption spectra, λ max and shoulder inflection/s of all the resolved bands.

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.

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 of the chromatogram with the same or different conditions.

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 is used to analyze 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).

Gas Chromatography (GC): It is used for authentication and quality control. The high selectivity of capillary columns enables separation of many volatile compounds simultaneously within comparatively short times. The disadvantage of GC is that this method is not

convenient for the analysis of samples which are thermolabile and non-volatile.

Hyphenated Techniques: Chromatographic separation techniques are coupled to various detection techniques such as mass spectrometry (MS), nuclear magnetic resonance (NMR), infrared spectroscopy (IR) etc. These techniques provide information about the structure of the compound present in chromatogram and provide higher sensitivity. 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): This technique is used to characterize wide variety of plant constituents ranging from small molecules to macromolecules such as peptides, proteins, carbohydrates and nucleic acids. Recent advances include electrospray, thermospray, and ionspray ionization techniques which offer unique advantages of high detection, sensitivity and specificity. Isotopes pattern can be detected by this technique.

Liquid chromatography – nuclear magnetic resonance (LC-NMR): The combination of chromatographic separation technique with NMR spectroscopy is one of the useful and time saving methods for the separation and structural elucidation of unknown compound mainly for the structural elucidation of light and oxygen sensitive substances.

Gas-chromatography- mass spectrometry (GC- MS): It is used for the analysis of volatile constituents of herbal medicines. Especially, the hyphenation with MS provides reliable information for the qualitative analysis of the constituents. The flow rate from capillary column is low so that the column output can directly be feed into ionization chamber of MS. The mass detector in GC is the Ion Trap Detector (ITD). In this instrument, ions are created from the eluted sample by chemical ionization and stored in a

radio frequency field; the trapped ions are then ejected from the storage area to an electron multiplier detector.

CONCLUSION:

Herbal formulations have reached extensive acceptability as therapeutic agents for treatment of several diseases. Herbal drug technology is used for converting botanicals materials into medicines, where standardization and quality control with proper integration of modern scientific techniques and traditional knowledge is important. The traditional approach towards standardization is insufficient for current herbal market and hence there is need for more advanced techniques for standardization. There are basically two techniques used for standardization these are chromatographic fingerprinting and DNA fingerprinting. The chromatographic fingerprinting is based on the chromatographic separation and identification of marker compound from other constituents. For these purpose TLC, HPTLC, HPLC, LC-MS, LC-NMR, GC-MS, GC-FID and SFC methods are used. The other method used is DNA fingerprinting. As the DNA fingerprint of genome remain the same irrespective of the plant part used while the phytochemical content will vary with the plant part used, physiology and environment, hence this is well established and highly precious method for standardization of herbal drug.

REFERNCES:

1. S.P. Joshi, K.R. Prabhakar and V.S. Gupta. Curr. Sci.1999, 77 (2) 234.
2. Rayaa, G. et al. Genetics. 2002,162, 1381-1388.
3. Nikam P, Kareparamban J, Jadhav A, Kadam V, Future Trends in Standardization of Herbal Drugs, Journal of Applied Pharmaceutical Science. 2012, 02(06), 38-44
4. Calixto JB, Braz J Med Biol Res. 2000,33, 179-189.
5. Patil P, Shettigar R, An Advancement of Analytical Techniques in Herbal Research, Journal of Advanced Scientific Research. 2010,01(01):8-14
6. Houghton P, Mukherjee P, Evaluation Of Herbal Medicinal Products, 1st ed. Pharmaceutical press; 2009.p. 396-421
7. Liang Y, Xie P, Chan K, Quality control of Herbal medicines, J Chromatogr B AnalytTechnol Biomed Life Sc. 2004,812(1-2): 53–70.
8. Rasheed A, Sravya B, Roja C, Review On Standardisation Of Herbal Formulation, International Journal Of Phytotherapy. 2012, 02(02),74-88
9. Srivastava S, Mishra N, Genetic Markers - A Cutting-Edge Technology in Herbal Drug Research, Journal of Chemical and Pharmaceutical Research. 2009, 01(01),1-18.
10. Purohit, Kakarani, Saluja. Concepts, Perspectives and Techniques Of Microbial Genetic and Protein Engineering in Pharmaceutical Biotechnology, Agrobios India, 2007.p.74-78.
11. DNA fingerprinting in plants, http://biosolutions.6te.net/articles_files/dna_fingerprinting.html .
12. Sambrook, J., Fritsch E.F., and Maniatis, T., Molecular cloning: a laboratory manual, cold spring harbor laboratory, cold spring harbor laboratory press, 2nd ed. New York. 1989; p1659.
13. Harish Vasudevan, DNA fingerprinting in the standardization of herbs and nutraceuticals, the science creative quaterly,2011,6. <http://www.scq.ubc.ca/dna-fingerprinting-in-the-standardization-of-herbs-and-nutraceuticals>.