AYURPUB.com
INTERNATIONAL AYURVEDA PUBLICATIONS

REVIEW ARTICLE ISSN 2456-0170

CURRENT TRENDS IN ANALYTICAL METHODS OF MEDICINAL PLANT DRUGS

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ABSTRACT

The herbal drug industry has grown by leaps and bounds in the past decade, with the sales of the over the counter drugs increased in billions. This has brought the safety and efficacy of these drugs into highlight. The growing interest of the West has also influenced the methods of standardization of these herbal drugs and medicines. Majority of medicines in Ayurveda contains herbal drugs as individual or in combinations. The analytical methods of standardizing an herbal drug are coming up with new advances each day. The procedures like chromatographic techniques, electrophoretic techniques, are also getting finer and today we can use these processes to our advantage to identify and authenticate the herbal drugs. The DNA barcoding, DNA molecular markers, Metabolomics are the stepping stones towards quality standardization. The collection and storage of the medicinal plants also should have standard procedures to avoid collection of unnecessary parts of the plants and contamination which occurs during collection and storage. The analytical methods used for standardization should be accurate, sensitive, and reproducible each time the experiment is repeated. The analytical methods used should also authentify and maintain the quality of the drug. Interdisciplinary research is the need of the hour to correlate complex relation between the efficacy of the drug and its analysis .Different branches of science like cell biology, organic chemistry, bioinformatics, statistics and many more will help to establish the efficacy of the herbal medicinal plant.

Keywords- Ayurveda, Analysis, Standardization, Chromatography, Electrophoresis, DNA Barcoding, Medicinal Plants

INTRODUCTION

The herbal drugs and medicinal plant products have been widely used for thousands of years in many parts of the world. Herbal drugs are the integral part of the Ayurvedic medicines. Medicinal plants constitute a source of raw material for both traditional and modern systems of medicine. In recent few decades, growth and popularity of herbal medicine and plant products have taken a significant share of the healthcare. Each country has its own set of guidelines to assess the quality control of the herbal medicine depending on the system they adapt to prepare herbal medicine. The importance of quality control and standardization of botanical products is of utmost concern for global acceptability of these drugs in the modern system of medicine.

Objectives- The objective of this article is to highlight the analytical methods used for

identification, authentification and purity of the plant drugs. Current analytical methods utilized for analysis are featured, with the mention of future upcoming techniques.

MATERIALS AND METHODS

Single herbs need to be checked for its quality, authenticity, adulteration, and other parameters. The performance and safety of the plants depend on its accuracy of the analytical methods. The analytical methods should be consistent, accurate, sensitive and reproducible every time we perform them.

The analysis of drug has been mentioned in the different pharmacopoeias; also different protocols have been mentioned .In India CCRAS has developed protocol for plant drug analysis[1]. Besides the traditional analytical methods newer discoveries helps us to analyze the plant drug more sensitively.

Chromatography-Chromatography is the science of separation in which the flow of the mobile phase promotes the separation of samples by differential migration. The separation depends on the differential affinities of solutes between two immiscible phases[2]. Depending on the state of the stationary phase and mobile phase there are different types of chromatography.

Thin Layer Chromatography-This technique is used for the detection, identification, quantification of the herbal drug phyto-constituents. HPTLC is a sophisticated form of TLC. But still TLC is a crude method which is used for preliminary drug analysis and helps to identify the solvent system which gives the maximum separation.Merits-The main advantage of this process is its simplicity, adaptability, simple sample preparation, and sensitivity of the test.This test can be performed on the crude extracts of the plant drugs to estimate the phytochemicals present in the drug.

High Performance Thin Layer Chromatography (HPTLC) –HPTLC is a more sensitive method of separation of the herbal drugs which uses less amount of sample and solvent but gives maximum, sharp and better separations of compounds[3]. This technique can also be used for stability studies and pharmaceutical testing. This technique complies with Good Lab Practices (GLP) and Good Analytical Laboratory Practices (GALP).The newer techniques used concomitantly are Forced Flow Planar Chromatography (FFPC) Rotation Planar Chromatography (RPC), Over-Pressured Layer Chromatography (OPLC), And Electro Planar Chromatography (EPC).Merits-Detection of adulterants in small amount of sample plant drugs can be done. It separates at a high speed and different samples can be analyzed concurrently[4].It may be a bit expensive but sample required is less, and accuracy of results are greater than TLC.

High Pressure Liquid Chromatography(HPLC)- It is essentially a form of column chromatography in which the stationary phase consists of small particle (3-50μm) packing contained in a column with a small bore (2-5mm), one end of which is attached to a source of pressurized liquid eluent (mobile phase). The three forms of high performance liquid chromatography most often used are ion exchange, partition and adsorption. It is the most extensive application in the analysis of herbal medicines which can analyze almost

all compounds. Reversed-phase (RP) columns are the most popular columns used in the analytical separation of herbal medicines. Merits- Used for analysis of chemical compounds in medicinal plants. Newer trends for HPLC- These are micellar electrokinetic capillary chromatography (MECC), high-speed counter-current chromatography (HSCCC), low-pressure size-exclusion chromatography (SEC), reversed-phase ion-pairing HPLC (RP IPC-HPLC), and strong anion-exchange HPLC (SAX-HPLC). They will provide new opportunities for good separation for some specific extracts of some herbal medicines[5].

Gas Chromatography (GC) -Gas chromatography (GC), also known as gas liquid chromatography (GLC), is a technique for separation of mixtures into components by a process which depends on the redistribution of the components between a stationary phase or support material in the form of a liquid, solid or combination of both and a gaseous mobile phase. Gas Chromatography is used mainly for separation of volatile contents of the drug. This technique is mostly hyphenated with Mass spectrometry. The essential oils obtained from the medicinal plants mostly contain antimicrobial activity[6] Identification and quantitation of volatile and semivolatile organic compounds in complex mixtures. It also determines the structure and molecular weights of the gaseous elements. Newer trends in Gas Chromatography- Static and dynamic headspace (SHS and DHS), High concentration capacity headspace techniques (HCCHS) [7].

Infrared Spectroscopy (NIR)- Near infrared (NIR) spectroscopy (800–2500 nm; 12,500–4000 cm^{-1}) has become one of the most attractive and used methods for analysis. It represents a noninvasive analytical tool allowing a fast and simultaneous qualitative and quantitative characterization of natural products and their constituents. NIR identifications are based on pattern recognition methods. The classification techniques can be divided into two categories: the unsupervised and the supervised ones. Unsupervised classification methods- Principal component analysis (PCA), Supervised classification methods- Linear discriminant analysis (LDA), Soft independent modelling of class analogy (SIMCA)[8]. The advantages of NIRS are many with rapidity of analysis being one of the most important. Thus NIRS combined with appropriate mathematical models and pattern recognition techniques allows analysis of a wide variety of sample types rapidly. Second, NIRS is a non-destructive technique which avoids complex sample preparation by chemical or physical processes. In fact, both solid and liquid samples in different types of packaging stored under different conditions can all be tested without complex pretreatment because of the better penetrability of fiber optics used in NIRS. Third, it provides acceptable accuracy in both qualitative and quantitative analysis to meet the requirements of QC and preliminary screening.[9]

Capillary Electrophoresis (CE)-It is versatile and powerful separation tool with high separation efficiency and selectivity when analyzing mixtures of low-molecular-mass components However, the fast development in capillary electrophoresis

causes improvement of resolution and throughput rather than reproducibility and absolute precision. It may have difficulty in reproducibility but the accuracy is of a good quality[10]. Merits-It is an efficient way for separation and identification of charged sample components, from simple inorganic ions to DNA.Different techniques of electrophoretic separation are used namely-Capillary zone electrophoresis (CZE),Capillary gel Electrophoresis(CGC),Capillary isoelectric focusing(CIEF).Having similarities with liquid chromatography this method of separation gives chemical fingerprints of the herbal medicines.

Ultraviolet Spectroscopy (UV_VIS)-It is the most common hyphenation technique mostly used with chromatography. It is a special tool in the plant spectral analysis. Colourless compounds can also be assessed with these spectra.200-400nm range of measurements are used for this while 200-700nm range is used for coloured compounds[11].

Differential Scanning Calorimeter(DSC)- DSC is a thermal analysis apparatus measuring how physical properties of a sample change, along with temperature against time.It has many applications including the analysis of proteins,carbohydrates and nucleic acids.The various types of DSC in use are-Conventional DSC, Micro electro mechanical Systems-DSC, Infrared-Heated DSC, Modulated-Temperature DSC, Gas Flow-Modulated DSC, Parallel-Nano DSC, Pressure Perturbation Calorimetry, Self-Reference DSC, And High-Performance DSC [12].

NMR (Nuclear Magnetic Resonance) Spectroscopy- NMR spectroscopy is nondestructive, selective, and capable of simultaneous detection of a great number of constituents. The identification by NMR provides on both major and minor components, with no restrictions relating to volatility, polarity, or the presence of specific chromophores. The method can be applied without knowledge of the composition of the sample. NMR spectra contain information about the identity of themolecules in the extracts and are useful to quantify the constituents. NMR spectroscopy coupled with chemometric tools has been successfully applied to the characterization of various herbs and plant extracts for quality control,authentication, determining geographical origin, and detecting adulteration of herbal drugs and extracts [13].

Mass Spectroscopy (MS)-Mass spectrometry is the most sensitive and selective method for molecular analysis and can yield information on the molecular weight as well as the structure of the molecule. Combining chromatography with mass spectrometry provides the advantage of both chromatography as a separation method and mass spectrometry as an identification method. In mass spectrometry, there is a range of methods to ionize compounds and then separate the ions. Mass Spectrometry based Proteomics- With the increase in the completion of the genome sequence of plant organisms, more attention has been focused on understanding the functions of genes on a genome widescale, not only the exact time and location of gene expression, but also which proteins and

under what conditions they express in certain cells, tissues, or organs. While the transcriptomic analysis can only provide us the spatial and temporal specificities or regulatory network relationships of gene expressions, proteome analysis can illustrate the post translational modifications of proteins, subcellular localizations, protein–protein interactions, and protein conformations.Application of MS is not only as a hyphenation technique ,in proteomics also [14].

DNA Barcoding Tecniques- The accurate identification of medicinal plants in relation to their purity and quality as well as safe application has become increasingly important.DNA barcoding is an established technique that uses the sequence diversity in short, standard DNA regions for species level identification. It is primarily used to identify known species by comparing their unique barcode sequences to reference sequences in public databases, as well as to facilitate species discovery. DNA barcoding provides a more rapid, subjective, and accurate identification compared with traditional methods. Thus, it has rapidly become a widely recognized tool for species identification. Provided a comprehensive evaluation of different DNA regions for the authentication of medicinal plants it has been found that the second internal transcriber spacer (ITS2) region could be used as a universal barcode for plant authentication. The ITS2 barcode has been tested recently in a wide range of taxa. It has been proven to be very effective for identifying medicinal plants [15].

DNA Molecular Markers- The use of chromatographic techniques and marker compounds to standardize botanical preparations has limitations because of their variable sources and chemical complexity. DNA-based molecular markers have utility in the fields like taxonomy, physiology ,embryology, genetics, etc. DNA-based techniques have been widely used for authentication of plant species of medicinal importance. Current focus on chemotype-driven fingerprinting and related techniques requires integration with genotype-driven molecular techniques so that an optimal characterization of botanical materials is possible.The different types of markers used are-Hybridization based,PCR based,and Sequencing based markers [16].

Metabolomics- The primary and secondary metabolites found in plant cells are the final recipients of biological information flow. In turn, their levels can influence gene expression and protein stability. Qualitative and quantitative measurements of these metabolites reflect the cellular state under defined conditions, and yield critical insights into the cellular processes that control the biochemical phenotype of the cell, tissue or whole organism[17]. Metabolomics can be defined as the area of research that strives to obtain complete metabolic fingerprints, to detect differences between them and to provide hypothesis to explain those differences. This area of research is still rapidly developing into a powerful tool in the study of all types of organisms[18].

DISCUSSION

The different analytical methods are discussed as some have different advantages over each other**.** According to Ayurveda the medicinal plant acts as a whole and not as a isolated compound, while the researchers are

busy isolating the active principles or compounds from the plants .The need of the hour is to understand the drug and its component as a whole and the way it works synergistically rather than the compounds in isolation. There is a thin line between the presence of the chemical compounds and efficacy of the plants. The records of the chemical compounds of the drug and from the region they are obtained has to be maintained for a period of time and this data could be analyzed statistically. This will give us the presence of compounds present during which time of the year and the area from which it is procured. Analytical methods help to identify the plant but efficacy can be judged by laboratory analysis. The analytical methods surely identify the chemical compounds present in the herbal drug. The accurate quantitative and qualitative assessment can be done by these techniques. The authentic drawback is the intricate relation between the chemical compounds, its quality, quantity and efficacy of the herbal drug. It is also very difficult to reproduce the results consistently. The chromatographic fingerprints certainly help a great deal in the identification and authentification of the drug, but the variation in amount of phytoconstituents can be dealt with by creating a database of these fingerprints and maintaining the records for years for ready reference. The different branches of science, like bioinformatics, biotechnology, organic chemistry, and statistics can all contribute towards a better understanding of these medicinal plant drugs. Interdisciplinary research, multicentric research is the necessity for a better understanding and utilization of these drugs.

CONCLUSION

The various analytical methods multiple applications. The need of study should be clear in the mind of the researcher to choose the correct analytical method. The methods have a few advantages over each other but they all are very sensitive, require small amount of sample and precise in nature. The recent advances in each technique will provide a finer and detailed information about the medicinal plant, its active principle or its extract. This information will certainly benefit to identify,authentify and give qualitative as well as quantitative information of the plant.

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Source of support: Nil,

Conflict of interest: None Declared

Cite this article as

Jyoti Brijesh Gavali : Current Trends in Analytical Methods of Medicinal Plant Drugs ayurpub 2016;I (3) 101-107