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A REVIEW ON MODIFICATION OF ANALYTICAL TECHNIQUES IN HERBAL RESEARCH

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ABSTRACT
As the demand and commercial value of the Herbal Medicines is increasing tremendously, assurance of safety, quality and efficacy of medicinal plants and herbal products is becoming a crucial issue. The need of the hour is to develop a systematic approach and well-designed methodologies for the standardization of herbal raw materials and herbal formulations. Standardization methods should take into consideration all aspects contributing to the quality of the herbal drugs. Herbal Medicines are composed of many constituents and are therefore very capable of variation. Hence it is very important to obtain reliable chromatographic fingerprints that represent pharmacologically active and chemically characteristic components of the Herbal Medicine. The information generated based on this chromatographic pattern 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. Several analytical techniques have been developed for obtaining fingerprinting profiles of the herbal medicines and have assured to be a valuable tool for proving constant composition of herbal preparations by establishing relevant criteria for uniformity. This paper deals with the advanced extraction as well as chromatographic techniques with the help of which qualitative and quantitative evaluation of Herbal Medicines and formulations can be carried out. The advancement of analytical techniques is thus bringing a new era of development which will serve as a rapid and tool in the herbal research thereby allowing the manufacturers to set quality standards and specifications so as to seek marketing approval from regulatory authorities.
KEYWORDS: Standardization, Herbal Medicine, Chromatographic fingerprinting, modification

INTRODUCTION

Herbal drugs have been used since ancient times as medicines for the treatment of a range of diseases. Medicinal plants have played a key role in world health. In spite of the great advances observed in modern medicine in recent decades, plants still make an important contribution to health care[1]. The recent global resurgence of interest in herbal medicines has led to an increase in the demand for them. The need of the hour is to evolve a systematic approach and to develop well-designed methodologies for the standardization of herbal raw materials and herbal formulations. Traditional systems of medicine are used for centuries all over the world. According to one estimate, 80 % of the world population still depends on herbal products for their primary healthcare needs. The toxic side effect of the drugs of modern medicine and the lack of medicines for many chronic ailments has led to the re-emergence of the herbal medicine, with possible treatments for many health problems[2]. They have stood the test of time for their safety, efficacy, cultural acceptability and lesser side effects. The chemical constituents present in them are a part of the physiological functions of living flora and hence they are believed to have better compatibility with the human body[3].

Most diseases, like diabetes, heart diseases, cancer, and psychiatric disorders, are multifactorial and hence need therapeutic intervention at more than one level. Plants with complex phytochemical mixtures have advantage over single molecules in treating such diseases, with an added advantage of being devoid of toxic side effects[2].

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. The herbal raw material is 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 xenobiotics. World Health Organization (WHO) stresses the importance of the qualitative and quantitative methods for characterizing the samples, quantification of the biomarkers and/ or chemical markers and the fingerprint profiles. If a principle active component is known, it is most logical to quantitate this compound. Where active ingredients contributing to therapeutic efficacy are known botanical preparations should be standardized to these compounds. Where the active ingredients are not yet known a marker substance which should be specific for the botanical could be chosen for analytical purpose[4]. The advancements in modern methods of analysis and the development of their application have made it possible to solve many of these problems. Extremely valuable are techniques like High-Performance Thin-layer Chromatography (HPTLC), Gas Chromatography (GC), Mass Spectrometry (MS), High-Performance Liquid Chromatography (HPLC), LC-MS, and GC-MS. Starting from sourcing of the raw material, standardization, preparation of the extracts, to formulation of the extracts into suitable dosage form, the problems vary with each plant species and part of the plant that is being used. At each and every step, phytochemical profiles have to be generated and a multiple-marker-based standardization strategy needs to be adopted to minimize batch-to-batch variation and to maintain quality and ensure safety and efficacy[1].

Phytochemical Standardization

Methods of standardization should take into consideration all aspects that contribute to the quality of the herbal drugs, namely correct identity of the sample, organoleptic evaluation, pharmacognostic evaluation, volatile matter, quantitative evaluation (ash values, extractive values), phytochemical evaluation, test for the presence of xenobiotics, microbial load testing, toxicity testing, and biological activity. Of these, the phytochemical profile is of special significance since it has a direct bearing 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 compound/s would serve as an additional parameter in assessing the quality of the sample.

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

1. Preliminary testing for the presence of different chemical groups.
2. Quantification of chemical groups of interest (e.g., total alkaloids, total phenolics, total triterpenic acids, total tannins).
3. Establishment of fingerprint profiles.
4. Multiple marker-based fingerprint profiles.
5. Quantification of important chemical constituents.[1]

ADVANCES IN EXTRACTION OF HERBALS

a. Supercritical Fluid Extraction

This is the most technologically advanced extraction system. Super Critical Fluid Extraction (SFE) involves use of gases, usually CO2, and compressing them into a dense liquid. This liquid is then pumped through a cylinder containing the material to be extracted. From there, the extract-laden liquid is pumped into a separation chamber where the extract is separated from the gas and the gas is recovered for re-use. Solvent properties of CO2 can be manipulated and adjusted by varying the pressure and temperature that one works at. The advantages of SFE are, the versatility it offers in pinpointing the constituents you want to extract from a given material and the fact that your end product has virtually no solvent residues left in it (CO2 evaporates completely). The downside is that this technology is quite expensive. There are many other gases and liquids that are highly efficient as extraction solvents when put under pressure.

Coupled SFE-SFC

System in which a sample is extracted with a supercritical fluid which then places the extracted material in the inlet part of a supercritical fluid chromatographic system. The extract is than chromatographed directly using supercritical fluid.

Coupled SFE-GC and SFE-LC

System in which a sample is extracted using a supercritical fluid which is then depressurized to deposit the extracted material in the inlet part or a column of gas or liquid chromatographic system respectively. SFE is characterized by robustness of sample preparation, reliability, less time consuming, high yield and also has potential for coupling with number of chromatographic methods.[1]

b. Microwave-Assisted Extraction

An innovative, microwave-assisted solvent-extraction technology known as Microwave-Assisted Processing (MAP) offers many advantages over conventional methods. Applications include the extraction of high-value compounds from natural sources including phytonutrients, nutraceutical and functional food ingredients and pharmaceutical actives from biomass. Compared to conventional solvent extraction methods, MAE technology offers some combination of the following advantages:

1. Improved products, increased purity of crude extracts, improved stability of marker compounds, possibility to use less toxic solvents;
2. Reduced processing costs, increased recovery and purity of marker compounds, very fast extraction rates, reduced energy and solvent usage.

With microwaves drive extraction as opposed to diffusion, very fast extraction rates and greater solvent flexibility is possible. Many variables, including the microwave power and energy density, can be tuned to deliver desired product attributes and optimize process economics. The process can be customized to optimize for commercial/cost reasons and excellent extracts are produced from widely varying substrates. Examples include, but are not limited to, antioxidants from dried herbs, carotenoids from single cells and plant sources, taxanes from taxus biomass, essential fatty acids from microalgae and oilseeds, phytosterols from medicinal plants, polyphenols from green tea, flavor constituents from vanilla and black pepper, essential oils from various sources, and many more.[2]

c. Solid Phase Extraction

This involves sorption of solutes from a liquid medium onto a solid adsorbent by the same mechanisms by which molecules are retained on chromatographic stationary phases. These adsorbents, like chromatographic media, come in the form of beads or resins that can be used in column or in batch form. They are often used in the commercially available form of syringes packed with medium (typically a few hundred milligrams to a few grams) through which the sample can be gently forced with the plunger or by vacuum. Solid phase extraction media include reverse phase, normal phase, and ion-exchange media. This is method for sample purification that separates and concentrates the analyte from solution of crude extracts by adsorption onto a disposable solid-phase cartridge. The analyte is normally retained on the stationary phase, washed and then evaluated with different mobile phase. If an aqueous extract is passed down a column containing reverse phase packing material, everything that is fairly nonpolar will bind, whereas everything polar will pass through.

Chromatographic Fingerprinting and Marker Compound Analysis

A chromatographic fingerprint of an Herbal Medicine (HM) is a chromatographic pattern of the extract of some common chemical components of pharmacologically active and or chemical characteristics. This chromatographic profile should be featured by the fundamental attributions of "integrity" and "fuzziness" or "sameness" and "differences" so as to chemically represent the HM investigated. It is suggested that with the help of chromatographic fingerprints obtained, the authentication and identification of herbal medicines can be accurately conducted (integrity) even if the amount and/or concentration of the chemically characteristic constituents are not exactly the same for different samples of this HM (hence, "fuzziness") or, the chromatographic fingerprints could demonstrate both the "sameness" and "differences" between various samples successfully. Thus, we should globally consider multiple constituents in the HM extracts, and not individually consider only one and/or two marker components for evaluating the quality of the HM products. However, in any HM and its extract, there are hundreds of unknown components and many of them are in low amount. Moreover, there usually exists variability within the same herbal materials. Hence it is very important to obtain reliable chromatographic fingerprints that represent pharmacologically active and chemically characteristic components of the HM.[6]

In the phytochemical evaluation of herbal drugs, TLC is being employed extensively for the following reasons: (1) it enables rapid analysis of herbal extracts with minimum sample clean-up requirement, (2) it provides qualitative and semi quantitative information of the resolved compounds and (3) it enables the quantification of chemical constituents. Fingerprinting using HPLC and GLC is also carried out in specific cases.

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, λmax 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. HPLC fingerprinting includes recording of the chromatograms, retention time of individual peaks and the absorption spectra (recorded with a photodiode array detector) with different mobile phases. Similarly, GLC is used for generating the fingerprint profiles of volatile oils and fixed oils of herbal drugs. Furthermore, the recent approaches of applying hyphenated chromatography and spectrometry such as High-Performance Liquid Chromatography–Diode Array Detection (HPLC–DAD), Gas Chromatography– Mass Spectroscopy (GC–MS), Capillary Electrophoresis- Diode Array Detection (CE-DAD), High-Performance Liquid Chromatography–Mass Spectroscopy (HPLC–MS) and High-Performance Liquid Chromatography–Nuclear Magnetic Resonance Spectroscopy (HPLC–NMR) could provide the additional spectral information, which will be very helpful for the qualitative analysis and even for the on-line structural elucidation.[3]

ADVANCES IN CHROMATOGRAPHIC TECHNIQUES

1. Liquid Chromatography

Preparative High Performance Liquid Chromatography

There are basically two type of Preparative HPLC. One is low pressure (typically under 5 bar) traditional PLC, based on the use of glass or plastic columns filled with low efficiency packing materials of large particles and large size distribution. A more recent form PLC, Preparative High Performance Liquid Chromatography (Prep.HPLC) has been gaining popularity in pharmaceutical industry. In preparative HPLC (pressure >20 bar), larger stainless steel columns and packing materials (particle size 10-30 µm are needed. The examples of normal phase silica columns are Kromasil 10 µm, Kromasil 16 µm, Chiralcel AS 20 µm whereas for reverse phase are Chromasil C18, Chromasil C8,YMC C18. The aim is to isolate or purify compounds, whereas in analytical work the goal is to get information about the sample. Preparative HPLC is closer to analytical HPLC than traditional PLC, because its higher column efficiencies and faster solvent velocities permit more difficult separation to be conducted more quickly. In analytical HPLC, the important parameters are resolution, sensitivity and fast analysis time whereas in preparative HPLC, both the degree of solute purity as well as the amount of compound that can be produced per unit time i.e. throughput or recovery are important. This is very important in pharmaceutical industry of today because new products (Natural, Synthetic) have to be introduced to the market as quickly as possible. Having available such a powerful purification technique makes it possible to spend less time on the synthesis conditions.[7]

Liquid Chromatography- Mass Spectroscopy (LC-MS) In Pharmaceutical industry LC-MS has become method of choice in many stages of drug development. Recent advances includes electro spray, thermo spray, and ion spray ionization techniques which offer unique advantages of high detection sensitivity and specificity, liquid secondary ion mass spectroscopy, later laser mass spectroscopy with 600 MHz offers accurate determination of molecular weight proteins, peptides. Isotopes pattern can be detected by this technique.[4]

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 structure 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.[8]

2. Gas chromatography

a. Gas Chromatography Fourier Transform Infrared spectrometry

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

b. Gas Chromatography-Mass Spectroscopy

GC equipment can be directly interfaced with rapid scan mass spectrometer of various types.

The flow rate from capillary column is generally low enough that the column output can be fed directly into ionization chamber of MS. 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 ions trap detector is remarkably compact and less expensive than quadrapole instruments. GC-MS instruments have been used for identification of hundreds of components that are present in natural and biological system.[5]

FUTURE OF HERBAL DEVELOPMENT

The quality of herbal substances, herbal preparations and herbal medicinal products is determined by the quality of the starting plant material, development, in-process controls, GMP controls, and process validation, and by specifications applied to them throughout development and manufacture. Specifications and acceptance criteria used to assure the quality of the herbal substances/preparations and herbal medicinal products at release and during the shelf life. All of the considerations listed above are necessary to ensure consistent production of herbal substances/preparations and herbal medicinal products of high quality. Thus to meet these requirements advanced analytical techniques like hyphenated chromatography and spectrometry would be helpful for detailed qualitative and quantitative analysis of herbal medicinal products so as to minimize batch to batch variation and assure safety, efficacy, quality and acceptability of the products.

CONCLUSION

Thus with the current advancement in techniques and new approach towards research, the herbal industry today is showing a serious shift in the paradigm worldwide. The need of high-technology oriented applications has given rise to investigate and offer highly advanced hyphenated techniques which will serve as a rapid and unambiguous tool in the herbal research and will also benefit the entire pharmaceutical.

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