

Devi Datt Joshi

Herbal Drugs and Fingerprints

Evidence Based Herbal Drugs

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Foreword



Globally the demand is increasing for medicines, pharmaceuticals, tonics, cosmetics and other plant based products. India is a major player in this area along with China, which is the world leader in the production, consumption and export of herbal products as well as raw materials. The current interest in the commercial production of these preparations and products has put tremendous pressure on the supply of raw materials. The burgeoning gap between availability of bioresources and demand on one hand and between commercial demand and supply on the other has led to use of both inappropriate (e.g., unsustainable collection from the wild, well beyond natural regeneration) and spurious practices (e.g., collection of raw material without any consideration of quality, deterioration of quality due to poor storage, adulteration or even substitution by a different part of the same plant or from an altogether different plant source and/or even material being spiked with synthetics). Such a situation calls for proper guiding material and techniques for fast and easy determination of genuineness of crude materials and for quality assurance in respect of herbal products. Fortunately literature is now becoming available, albeit slowly, that provides the much needed know how for determining the reliability of the raw material used along with the plant source and information on the availability, production and quality requirements.

Herbals are derived from whole plants or plant parts and used to prevent, relieve and treat illness. Since antiquity they are valued for medicinal, aromatic, nutritional and even rejuvenating qualities, and have played crucial role in maintaining the well being and vitality of human body, and for alleviating human suffering across civilizations.

The complex metabolic pathway of the human body can be adversely affected by the use of non-standardized herbals and herbal drugs. Good manufacturing practices, among others, insist on use of standardized protocols during the processing of raw materials (collection, transport, storage) and production of the final products or drugs. Standardized herbals and herbal drugs contain active ingredients, present in the naturally occurring plant source, in certain quantity and the proportion between different constituents/active principles is a key quality parameter for the efficacy of the product. It is in this regard that the modern tools and techniques of analysis provide vital support and required evidence. The present compilation is a unique and welcome attempt by the author to bring together current tools of finger printing and analyses for various classes of phytochemicals and natural compounds. The book *Herbal Drugs and Fingerprints* should be able to bridge an important gap, and would be useful for herbal healthcare professionals, scientists and researchers as well as to industries based on the herbals.

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Preface

Herbal drugs are time tested and valuable resource for healing, even today, globally. As the demand and commercial value of these drugs is increasing tremendously, assurance of safety, quality, and efficacy of medicinal plants and produces is becoming a crucial issue. The need of the hour is to develop an evidence-based market of herbal drug raw materials and herbal drugs. Herbal drugs are composed of many constituents and are therefore very capable of variation; hence, it is very important to obtain reliable fingerprints that bear pharmacologically active and chemically characteristic components of the herbal drug. The information generated based on fingerprints 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 drugs and have assured to be a valuable tool for proving constant composition of herbal preparations by establishing relevant criteria for uniformity. These fingerprints deal with the advanced extraction as well as analytical techniques with the help of which qualitative and quantitative evaluation of herbal drugs and formulations can be carried out and serve as a rapid and unambiguous tool in the herbal research thereby allowing the manufacturers to set quality standards, specifications, and seek marketing approval from regulatory authorities. Quality control of herbal drugs is a tedious and difficult job as herbs differ from that of the conventional drugs, so some innovative methods are into practice for the sake of quality assessment. Fingerprint analysis using chromatography has become the most potent tool for quality control of herbal drugs because of its simplicity and reliability, for identification, authentication, and adulteration. These fingerprints have global acceptance by regulatory authorities to determine authenticity and reliability of chemical constituents of herbal drugs and formulations.

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. In case a principle active component is known, it is most logical to develop fingerprints for the same as main evidence, on the whole fingerprint

profile, whereas the active ingredients contributing to therapeutic efficacy are not yet known as marker substance should be specific for the medicinal herb for evidence-based fingerprints. 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 TLC, HPTLC, HPLC, GC, MS, LC–MS, GC–MS, NMR, LC–MS–NMR, and GC–MS–NMR. Starting from raw material, processing, and formulation into suitable dosage form, claims are deciphered by fingerprints at every step. At each and every step, fingerprints have to be generated and a multiple-marker-based fingerprints strategy needs to be adopted to minimize batch-to-batch variation and to maintain quality and ensure safety and efficacy.

In order to have a good coordination between the quality of raw materials, in-process materials and the final products, it has become essential to develop reliable, specific, and sensitive fingerprints using a combination of classical and modern instrumental method of analysis. It also encompasses the entire field of study from birth of a plant to its clinical application, including herbal drug preparation of a defined content of a constituent or a group of substances with known therapeutic activity, respectively, by adding excipients or by mixing herbal drugs or herbal drug preparations.

Sometimes, same species of medicinal herb has different pharmacological activities depending on its area of origin, the methods used to extract it, and the part of the herb used, among other factors. After establishing differences and similarities in fingerprints among different samples of the same type of herb, we can attempt to have an answer as to why these different samples give different pharmacological activities, ultimately explaining the “cooperative effects” of components and effectively controlling the quality of these drugs during production, using hyphenated techniques. Fingerprints by hyphenated techniques lead to high robustness with all information along structural characterization.

The sole aim of this book is to collect all the fingerprint techniques for herbal drugs at one place, by various case studies globally for different reasons, using latest innovations, so that it may be helpful in the development of evidence-based herbal drugs and new herbal drug development.

D.D. Joshi

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Herbal Drugs: A Review on Practices

The use of herbals as drug is older than recorded history, as mute witness to this fact are marshmallow (*Althaea officinalis*) root, hyacinth (*Hyacinthus orientalis*), and yarrow (*Achillea millefolium*), found carefully tucked around the bones of a Stone Age man in Iraq. These herbs are used as demulcent, diuretic, and common cold remedy, respectively, even today. Ayurveda and TCM are two great living traditions of the world which are related to herbal healthcare. Every continent has its folk system for healing and caring, and a few are still recognized by the state healthcare agencies, in different countries. King Hammurabi of Babylon (1800 BC) had prescribed the use of mint for digestive disorders; now, it has been established by modern science that peppermint indeed relieves nausea and vomiting by mildly anesthetizing the lining of the stomach. The knowledge of herbal drugs was widely disseminated throughout Europe by the seventeenth century. Nicholas Culpeper had written “A Physical Directory” and “The English Physician,” the first manuals that a layperson could use for healthcare, and it is still widely referred and quoted. The first publication of the US Pharmacopoeia (1820) included an authoritative listing of herbal drugs, with descriptions of their properties, uses, dosages, and tests of purity. It was periodically revised and became the legal standard for medical compounds in 1906, but with development of synthetic chemistry, synthetic therapeutic ingredients gained preference, and herbal drugs became the secondary choice.

The synthetic therapeutic products could not be accepted more due to severe side effects, and there was relook for herbal therapy, and natural products were found as storehouse of different molecular designs, even beyond the human imagination. A few traditional remedies directly passed the modern molecular approach for treatment, for example, ephedrine, an active ingredient of *Ephedra*, is used in the commercial pharmaceutical preparations for the relief of asthma symptoms and other respiratory problems, as it helps the patient to breathe more easily, while *Ephedra* in TCM was in use since 2,000 years back to treat the same ailments. Foxglove (*Digitalis lanata*) leaves are known since 1775, till the date, as cardiac stimulant and keeps alive the millions of heart patients worldwide. The active ingredient has been identified as digoxin, a medicine of all pharmacopoeias.

There are over 750,000 plants on earth, but relatively, only a very few have been studied for healing. Modern pharmacology looks for one active ingredient and seeks to isolate it, with the exclusion of all others. Most of the research done on herbals is focused on identifying and isolating active ingredients, rather than studying the medicinal properties of whole herb. Herbalists, however, consider that the power of herbal lies in the interaction of all its ingredients. Used as a drug, herbals offer synergistic interactions between ingredients both known and unknown. FDA has categorized most of the herbals as food supplements or nutraceuticals, knowingly acknowledging the wisdom of centuries-old practices. The sole aim of this section is a review on the different approaches adopted globally, by different regimes, at different times, based on sociopolitical scenario to ensure for correct quantity and quality of herbal drugs to induce the desired therapeutic effects.

Herbal Drugs and Chromatographic Fingerprints

Chromatography is a technique for the separation of mixture of solutes brought about by the dynamic partition or distribution of dissolved or dispersed material between two immiscible phases, one of which is moving on the other. Every type of chromatography contains a mobile phase and a stationary phase. The moving phase may be liquid or gas. Chromatography, in its various forms, is used for concentrating/identification of component which is in dilution but has high commercial value (e.g., Taxol from *T. baccata*, digoxin from *D. lanta*, and forskolin from *C. forskohlii*). This is an extremely valuable technique for the separation, isolation, purification, and identification of components from the mixture. Chromatographic fingerprints of herbals and herbal drugs may be defined as the chromatographic pattern of pharmacologically active and or chemically characteristic constituents present in the extract, which is featured by the fundamental attributions of integrity and fuzziness with the reference or reference standard. Due to geo-climatic factors, there is high variability of chemical components, even in the same species, collected from different zones. As the therapeutic effect of herbals is based on interaction of numerous ingredients present on it, so different kinds of chromatographic fingerprint techniques for quality control of herbal drugs have gradually come into practice, such as TLC, HPTLC, HPLC, and CLC, for the purpose of species authentication, evaluation of quality and ensuring the consistency, and stability of herbal drugs and their related products.

In herbals, a large number of chemical components are involved, and many of them are in low concentration. Chromatographic instruments and experimental conditions are difficult to reproduce during real analysis resulting in baseline and retention time shifts from one chromatogram to another, and a few associated complications such as abnormal chromatograms and ghost peaks are additional difficulties during fingerprint development, so chemometric approaches such as variance analysis, peak alignment, correlation analysis, and pattern have been employed to deal with the chromatographic fingerprint. Many mathematical algorithms are used for data processing in chemometric approaches. The basic principles for this approach are variation determination of common peaks/regions and similarity comparison with similarity index and linear correlation coefficient. Similarity index and linear correlation coefficient can be used to compare common pattern of the

chromatographic fingerprints obtained. In general, the mean or median of the chromatographic fingerprints under study is taken as the target, and both are considered to be reliable. To facilitate the data processing, a “computer aided similarity evaluation” (CASE) software has been developed.

Currently, many hyphenated techniques with chromatography are in practices for quality assessment, authentication, and content quantification, and there is still lookout for further innovations. A brief on the utility of a few chromatographic techniques with latest update to develop fingerprints of herbals and herbal drugs is described in this section.

Herbal Drugs and Spectral Fingerprints

The relook on herbal healthcare has developed a challenge and demand to regulatory authorities and scientists to authenticate that products/produces have not been adulterated with contaminants or cheaper ingredients. Analytical authentication is an important quality criterion for efficacy and safety, as it is focused on specific molecular markers or on recording compositional profiles of the ingredients. Spectroscopic techniques, which are based on interaction between electromagnetic radiation and atoms/molecules of the sample, are very attractive tools due to simplicity, fast and easy mode of operation. Complex mixtures are analyzed with hyphenated chromatographic devices to cross validate the analysis. Electromagnetic spectrum is a classification of photons with various energies into different spectral regions. UV-Vis. spectroscopy is used when high-energy photons (wave length 200–400 nm) are absorbed by atoms/molecules of sample which causes electronic excitation. Visible wavelengths cover a range from 400 to 800 nm.

IR spectroscopy is an analytical technique used to identify organic, as well as some inorganic, materials in the herbals and products, in solid as well as liquid forms. Mid-infrared spectroscopy ($400\text{--}40,000\text{ cm}^{-1}$) is useful to identify a variety of adulteration problems using experimental and statistical methods. NIR ($4,000\text{--}14,000\text{ cm}^{-1}$) is another rapid, reliable, and nondestructive technique that is widely used for quality and processing control for the qualitative characterization of various products in exploring bulk material with little or no sample preparation. FTIR, an advanced form of IR, looks at the mid-infrared spectrum. The region between $1,500$ and 400 cm^{-1} is referred to as the fingerprint region. Absorption bands in this region are generally due to intramolecular phenomena and specify molecular composition and structure.

The m/z value of molecule and its elucidation is characteristic of mass spectrometry fingerprints while NMR for the environment of protons around it. LC-MS has become method of choice in many stages of drug development. LC-NMR improves speed and sensitivity of detection and found useful in the areas of pharmacokinetics, toxicity studies, drug metabolism, and drug discovery process. The identification of adulterants in a Chinese herbal medicine was done by LC-NMR technique. GC-MS instruments have been used for identification of large number of components present in natural and biological

systems. The identification and quantification of chemical constituents present in polyherbal oil formulations is carried out by GC–MS method.

For trace-level analysis, a combination of column liquid chromatography or capillary gas chromatography with a UV–Vis. or an MS has become the preferred approach to develop reliable fingerprints. Various hyphenated procedures used for the analysis of herbal drugs are HPLC–DAD, CE–DAD, GC–MS, LC–MS, HPLC–MS, HPLC–DAD–MS, and LC–DAD–MS. The data obtained from such hyphenated instruments are the so-called two-way data; say one way for chromatogram and the other way for spectrum, which could provide much more information than the classic one-way chromatography. A “total analysis device” has been recently demonstrated in the case of online HPLC–UV (DAD)–FTIR–NMR–MS analyses. In this section, efforts have been focused on to gather all related potential information based on important case studies, in summarized form, as a clue for any new problem, to develop validated fingerprints.