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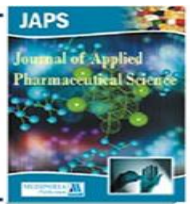

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Future Trends in Standardization of Herbal Drugs

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ABSTRACT

In recent years more people throughout world are turning to use medicinal plant products in healthcare system. World wide need of alternative medicine has resulted in growth of natural product markets and interest in traditional systems of medicine. 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. In order to prove constant composition of herbal preparations, adequate analytical methods the have to be applied such as photometric analysis, thin layer chromatography [TLC], high performance liquid chromatography [HPLC], and gas chromatography [GC], DNA Fingerprinting.

Keywords: Standardization, Chromatographic Fingerprinting, DNA Fingerprinting.

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 (Calixto *et al*., 2000). Natural products have been our single most successful source of medicines. Each plant is like factory capable of synthesizing unlimited number of highly complex and unusual chemical substances whose structures could otherwise escape the imagination forever (Kinghorn, 2002). There are at least 120 distinct chemical substances derived from plants that are considered as important drugs currently in use in the world, while several other drugs are simple synthetic modifications of the natural products (Farooqi, 2001). WHO has provided some terms related to herbal drugs, according to their definitions. **Herbal medicines** include *herbs, herbal materials, herbal preparations* and *finished herbal products.* In some countries herbal medicines may contain, by tradition, natural organic or inorganic active ingredients that are not of plant origin (e.g. animal and mineral materials).

Herbs include crude plant material, such as leaves, flowers, fruit, seeds, stems, wood, bark, roots, rhizomes or other plant parts, which may be entire, fragmented or powdered.

Herbal materials include, in addition to herbs, fresh juices, gums, fixed oils, essential oils, resins and dry powders of herbs. In some countries, these materials may be processed by various local procedures, such as steaming, roasting or stir-baking with honey, alcoholic beverages or other materials.

Herbal preparations are the basis for finished herbal products and may include comminuted or powdered herbal materials, or extracts, tinctures and fatty oils of herbal materials. They are produced by extraction, fractionation, purification, concentration, or other physical or biological processes. They also include preparations made by steeping or heating herbal materials in alcoholic beverages and/or honey, or in other materials.

Finished herbal products consist of herbal preparations made from one or more herbs. If more than one herb is used, the term "mixture herbal product" can also be used. Finished herbal products and mixture herbal products may contain excipients in addition to the active ingredients. However, finished products or mixture herbal products to which chemically defined active substances have been added, including synthetic compounds and/or isolated constituents from herbal materials, are not considered to be herbal (WHO guideline, 2000).

So that it is necessary to maintain reproducible efficacy and safety of phytopharmaceutical therefore if phytopharmaceuticals have to regarded as rational drug they should be standardized and pharmaceutical quality must be approved (Bauer *et al.,* 1993).

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 (Dixit *et al.,* 2008).

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. 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 xenobiotic (Dixit *et al.,* 2008). Standardization as defined by American Herbal Product association: "Standardization refers to the body of information and control necessary to product material of reasonable consistency. This achieved through minimizing the inherent variation of natural product composition through quality assurance practices applied to agricultural and manufacturing processes (Waldesch *et al.,* 2003). 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). Establishment of fingerprint profiles.
3. Multiple marker-based fingerprint profiles.
4. Quantification of important chemical constituents (Calixto *et al.,* 2000).

METHODS OF STANDARDIZATION

Phytotherapeutic agents are normally marketed as standardized preparations in the form of liquid, solid (powdered extract), or viscous preparations. They are prepared by maceration, percolation or distillation (volatile oils). Ethanol, water, or mixtures of ethanol and water are used for the production of fluid extracts. Solid or powered extracts are prepared by evaporation of the solvents used in the process of extraction of the raw material. Some phytotherapeutic agents are greatly concentrated in order to improve their therapeutic efficacy (Schulz *et al.,* 1996).

Approach towards standardization of herbal drug

Conventional methods for standardization of herbal formulation

Standardization of herbal raw drugs include passport data of raw plant drugs, botanical authentification, microscopic & molecular examination, identification of chemical composition by various chromatographic techniques and biological activity of the whole plant (Patel *et al.,* 2006). Macroscopic and microscopic evaluation and chemical profiling of the herbal materials for quality control and standardization have been in use for standardization. Macroscopic identity of medicinal plant materials is based on sensory evaluation parameters like shape, size, colour, texture, odour and taste while microscopy involves comparative microscopic inspection of powdered herbal drug. Further, advances in microscope technology have increased the accuracy and

capabilities of microscopy as a mean of herbal crude material identification due to the implication of light and scanning electron microscopes (SEM) in herbal drug standardization (Bhutani, 2003).

Standardization of herbal formulation

Standardization of herbal formulation requires implementation of Good Manufacturing Practices (GMP) (WHO guideline, 1996) 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 (Mosihuzzaman *et al.,* 2008). Heavy metals contamination, Good Agricultural Practices (GAP) in herbal drug standardization are equally important. (Bauer, 1998).

Standardization of polyherbal formulations

Standardization is an important aspect for maintaining and assessing the quality and safety of the polyherbal formulation as these are combinations of more than one herb to attain the desire therapeutic effect (Sharma *et al*., 2009). Standardization minimizes batch to batch variation; assure safety, efficacy, quality and acceptability of the polyherbal formulations (Ahmad *et al*., 2006).

Guidelines for the standardization of herbal drugs

The guidelines set by WHO:

Botanical characters, sensory evaluation, foreign organic matter, microscopic, histological, histochemical assessment, quantitative measurements,

Physical and chemical identity, fingerprints chromatography, ash values, extractive values, moisture content, volatile oil and alkaloids tests, quantitative estimation protocols, c) Estimation of biological activity, the values of bitterness, astringency hemolytic index, a factor swelling, foaming index,

Detail-toxicity pesticides residues, heavy metals, microbial contamination as viable count total, pathogens such as *E. coli, Salmonalla, P. aeroginosa, S. aureus, Enterobacteriaceae,*

*M*icrobial contamination and radioactive contamination are followed (Shrikumar *et al.,* 2006).

Table 1: General Testing Parameters for Characterization and Standardization of Herbal Medicines.

Title	Testing Parameter	Guidelines
General data	Geographical Harvesting time Harvesting process Processing	Good Agricultural Practices (GAP)
Description		
Identity	Macroscopic Microscopic Chemical TLC fingerprints	According to Pharmacopoeias
Purity	Foreign matter Ash/Sulfated ash Content of extractable matter Water content	According to Pharmacopoeias
Assay	Constituents with known therapeutic activity (biomarker) Constituents with unknown therapeutic activity (marker substances) Titrimetric Photometric HPLC/GC/TLC	According to Pharmacopoeias
Contaminants	Pesticides Heavy metals Aflatoxins Microbiological purity Radioactivity	Ph. Eur. Recommended limits for herbal drugs (oct. 91) Regulation on aflatoxins (Nov. 90) Ph. Eur. 1997 Suppl. 1999

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.

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. 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.
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, λ 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. 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 (Liang *et al.,* 2004, Ong *et al.,* 2002).

HPTLC

HPTLC technique is widely employed in pharmaceutical industry in process development, identification and detection of adulterants in herbal product and helps in identification of pesticide content, mycotoxins and in quality control of herbs and health foods (Soni, et al, 2010) It has been well reported that several samples can be run simultaneously by use of a smaller quantity of mobile phase than in HPLC (Jianga *et al.,* 2010). It has also been reported that mobile phases of pH 8 and above can be used for HPTLC. Another advantage of HPTLC is the repeated detection (scanning) of the chromatogram with the same or different conditions. Consequently, HPTLC has been investigated for simultaneous assay of several components in a multi-component formulation (Thoppil *et al.,* 2001). With this technique, authentication of various species of plant possible, as well as the evaluation of stability and consistency of their preparations from different manufactures.

Various workers have developed HPTLC method for phytoconstituents in crude drugs or herbal formulations such as bergenin, catechine and gallic acid in *Bergenia cilliata* and *Bergenia lingulata* (Dhalwal *et al*., 2008).

HPLC

Preparative and analytical HPLC are widely used in pharmaceutical industry for isolating and purification of herbal compounds**.** There are basically two types of preparative HPLC: low pressure HPLC (typically under 5 bar) and high pressure HPLC (pressure >20 bar) (Chimezie *et al.,* 2008). The important parameters to be considered are resolution, sensitivity and fast analysis time in analytical HPLC whereas 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 in preparative HPLC (Rao *et al*., 2009). 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. 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 (Bhutani, 2000; Marston, 2002; Brandt *et al.,* 2002).

Liquid Chromatography- Mass Spectroscopy (LC-MS)

LC-MS has become method of choice in many stages of drug development (Lee,1999). Recent advances includes electrospray, thermospray, and ionspray 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 (Bhutani, 2000).

Liquid Chromatography- Nuclear Magnetic Resonance (LC-NMR)

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 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 (Patil et al, 2010).

GAS CHROMATOGRAPHY (GC-MS)

GC equipment can be directly interfaced with rapid scan mass spectrometer of various types. GC and GC-MS are 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 (Guo *et al*., 2006 and Teo *et al.,* 2008).

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 (Sharma, 2009).

GC-FID

A number of detectors are used in gas chromatography. The most common are the flame ionization detector (FID) and the thermal conductivity detector (TCD). Coupling capillary column gas chromatographs with Fourier Transform Infrared Spectrometer provides a potent means for separating and identifying the components of different mixtures (Sharma).

Both are sensitive to a wide range of components, and both work over a wide range of concentrations. While TCDs are essentially universal and can be used to detect any component other than the carrier gas (as long as their thermal conductivities are different from that of the carrier gas, at detector temperature), FIDs are sensitive primarily to hydrocarbons, and are more sensitive to them than TCD. However, an FID cannot detect water. Both detectors are also quite robust. Since TCD is non-destructive, it can be operated in-series before an FID (destructive), thus providing complementary detection of the same analytes (Patra *et al*., 2010).

SUPERCRITICAL FLUID CHROMATOGRAPHY (SFC)

Supercritical fluid chromatography is a hybrid of gas and liquid chromatography that combines some of the best features of each. SFC permits the separation and determination of a group of compounds that are not conveniently handled by either gas or liquid chromatography. SFC has been applied to a wide variety of materials including natural products, drugs, food and pesticide. (Matthew et al, 2006). These compounds are either nonvolatile or thermally labile so that GC procedures are inapplicable or contain no functional group that makes possible detection by the spectroscopic or electrochemical technique employed in LC (Patil *et al.,* 2010).

DNA FINGERPRINTING

DNA analysis has been proved as an important tool in herbal drug standardization. This technique is useful for the identification of phytochemically indistinguishable genuine drug from substituted or adulterated drug. It has been reported that DNA fingerprint genome remain the same irrespective of the plant part used while the phytochemical content will vary with the plant part used, physiology and environment (Shikha et al,2009).

Deoxyribonucleic acid (DNA) is the fundamental building component of all living cells. Our characteristics, traits and physical features are determined by the specific arrangement of DNA base-pair sequences in the cell. It is this distinct arrangement of adenine, guanine, thymine and cytosine (called DNA nucleotides) that regulates the production of specific proteins and enzymes via the *Central Dogma Theory*. Central Dogma theory can be defined as the fundamental theory of molecular biology that genetic information flows from DNA to RNA to proteins (Breithaupt, 2003). This concept of fingerprinting has been increasingly applied in the past few decades to determine the ancestry of plants, animals and other microorganisms. Genotypic characterization of plant species and strains is useful as most plants, though belonging to the same genus and species, may show considerable variation between strains. Additional motivation for using DNA fingerprinting on commercial herbal drugs is the availability of intact genomic DNA from plant samples after they are processed. Adulterants can be distinguished even in processed samples, enabling the authentication of the drug (Mihalov *et al.*, 2000).

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 (Lazarowych et al, 1998).

GENETIC MARKER

A *genetic marker* 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), or a long one, like minisatellites.

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)

They can be further categorized as dominant or co-dominant. Dominant markers allow for analyzing many loci at one time, e.g. RAPD. A primer amplifying a dominant marker could amplify at many loci in one sample of DNA with one PCR reaction. Co-dominant markers analyze one locus at a time. A primer amplifying a co-dominant marker would yield one targeted product (Raya *et al*., 2002).

ROLE OF GENETIC MARKER IN HERBAL DRUG TECHNOLOGY

Genetic variation/genotyping

It has been well documented that geographical conditions affect the active constituents of the medicinal plant and hence their activity profiles. Many researchers have studied geographical

variation at the genetic level. Estimates of genetic diversity are also important in designing crop improvement programmes for management of germ plasm and evolving conservation strategies. RAPD-based molecular markers have been found to be useful in differentiating different accessions of neem collected from different geographical regions (Khanuja, 2002).

Germplasm analysis to study genetic diversity is another important area in which a lot of efforts have been put in. Fingerprinting of crops like rice wheat, chickpea, pigeon pea, pearlmillet etc is being carried out extensively (Khanuja, 2002; Ramakrishna *et al.,* 1994).

Authentication of medicinal plants

DNA-based techniques have been widely used for authentication of plant species of medicinal importance. This is especially useful in case of those that are frequently substituted or adulterated with other species or varieties that are morphologically and/or phytochemically indistinguishable (Srivastava *et al.,* 2009). Dried fruit samples of *Lycium barbarum* were differentiated from its related species using RAPD markers .The RAPD technique has also been used for determining the components of a Chinese herbal prescription, yu-pingfeng san. In this study the presence of three herbs (*Astragalus membanaceus* (Fisch.) Bge, *Ledebouriella seseloides* Wolff and *Atractylodes macrocephala* Koidz) in the formulation have been detected using a single RAPD primer (McCouch *et al.,* 1988).

Detection of adulteration/substitution

Sequence characterized amplified region (SCAR), AP–PCR, RAPD and RFLP have been successfully applied for differentiation of these plants and to detect substitution by other closely related species**.** e.g. *P. ginseng* is often substituted by *P. quinquefolius* (American ginseng) (Lau et al.,2001).

Medicinal plant breeding

ISSR–PCR has been found to be an efficient and reliable technique for the identification of zygotic plantlets in citrus interploid crosses. Molecular markers have been used as a tool to verify sexual and apomictic offspring of intraspecific crosses in *Hypericum perforatum*, a well known antihelminthic and diuretic.An attempt has been made towards marker-assisted selection of fertile clones of garlic with the help of RAPD markers. RAPD markers have been successively used for selection of micropropogated plants of *Piper longum* for conservation (Ratnaparkhe *et al.,* 1995; Shaw et al, 1995).

Quality control and standardization of medicinal plant materials

To ensure efficacy, selection of the correct chemo type of the plant is necessary even when there are many known chemotypes of a plant species, selection of the right chemo type to which clinical effects are attributed is difficult. DNA markers are reliable for informative polymorphism as the genetic composition is unique for each species and is not effected by age, physiological condition as well as environmental factors .DNA can be extracted from fresh or dried organic tissue of the botanical material; hence the physical form of the sample for assessment does not restrict detection. Various types of DNA-based molecular techniques are utilized to evaluate DNA polymorphism. These are hybridization-based methods polymerase chain reaction (PCR) based methods and sequencing-based methods (Cai *et al.,* 1999).

CONCLUSION

The Indian herbal industry is growing in a tremendous rate. More number of herbal products is arrived in the market. The safety and efficacy of herbal products are dependent upon the standardization of these herbal drugs. 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.

REFERENCES

Ahmad I, Aqil F, Owais M. Turning medicinal plants into drugs. Modern Phytomed 2006; 384:67-72

Bauer R, Czygan F-C, Franz G, Ihrig M, Nahrstedt A, Sprecher E. Demands on the quality of rationally applicable phytopharmaceuticals (in German).Dtsch Apoth Ztg. 1993; 133:4105–4108

Bauer R, Czygan F-C, Franz G, Ihrig M, Nahrstedt A, Sprecher E. Pharmaceutical quality, standardization and normalization of phytopharmaceuticals (in German). Z Phytother. 1994, 15:82–91

Bauer R. Quality criteria and standardization of phytopharmaceuticals: Can acceptable drug standard can be achieved .J Drug Inform 1998; 32:101-110

Bhutani KK, Finger-Printing of Ayurvedic Drugs, The Eastern Pharmacist, 2000; 507: 21-26

Bhutani KK. Herbal medicines enigma and a challenge for science and guidelines for new initiatives. J Nat Prod 2003; 19(1): 3-8.

Brandt A, Schering AG, Kueppers S, Practical Aspects of Preparative HPLC in Pharmaceutical and Development Production. (www.lcgceurope.com), 2002; 2-5

Breithaupt, H. (2003) Back to the roots EMBO Rep 4(1): 10-12.

Cai Z. H., Li P., Dong T. T. and Tsim K. W**.,** Molecular diversity of 5S-rRNA spacer domain in Fritillaria species revealed by PCR analysis., Planta Med 1999, 65: 360-64.

Calixto J.B, Barz J., Efficacy, safety, quality control, marketing and regulatory guidelines for herbal medicines (phytotherapeutic agents), Med Biol Res, 2000, 33: 179-189.

Chimezie A, Ibukun A, Teddy E, Francis O. HPLC analysis of nicotinamide, pyridoxine, riboflavin and thiamin in some selected food products in Nigeria. Afr J Pharm Pharmacol 2008; 2(2):29-36

Dhalwal K., Sindhe V.M., Biradar Y.S., and Mahadik K.R.. Simultaneous quantification of bergenin, catechine, and gallic acid from Bergenia ciliate and Bergenia lingulata by useing thin-layer chromatography, J. Food. Comp. Anal 21: 2008 .496-500.

Dixit V.K., Yadav N.P. Recent approaches in herbal drug standardization. Integr Biol, 2008; 2 (3): 195-203.

Farooqi A.A. and Sreeramu B.S. Cultivation of medicinal and aromatic crops. University press, Delhi, 2001, 9-10.

General guidelines for methodologies on research and evaluation of traditional medicine. Geneva, World Health Organization, 2000 (WHO/EDM/TRM/2000.1)

Guo F.Q., Huang L.F., Zhou S.Y., Zhang T.M., Liang Y.Z., Comparison of the volatile compounds of Atractylodes medicinal plants by headspace solid-phase microextraction-gas chromatography–mass spectrometry.Anal. Chim. Acta 570: (2006) 73-78 .

Jianga Y., David B., Tu P., Barbin Y.. Recent analytical approaches in quality control of traditional Chinese medicines—A review, Anal. Chim. Acta 2010, 657, 9–18.

Kartik Chandra Patra, Surendra K. Pareta, Ranjit K. Harwansh, K. Jayaram Kumar. Traditional Approaches towards Standardization of Herbal Medicines. Journal of Pharmaceutical Science and Technology 2010; 2 (11):372-379.

Khanuja SPS, Shasany AK, Aruna V, Darokar MP, Kalra A, Bahl JR, Bansal, RP RAPD marking of three Pelargonium graveolens genotypes with chemotypic differences in oil quality., J. Med. Aromat. Plant Sci. 2002, 24: 729–32.

Kinghorn A.D. The role of pharmacognosy in modern medicine, Expert Opin. Pharmacother. 2002, 3(2) 77-79.

Lau.D. T., Shaw P. C,., Wang J., and Shaw P. C., Authentication of medicinal Dendrobium species by the internal transcribed spacer of ribosomal DNA, Planta Med.,2001, 67, 456–460.

Lazarowych NJ, Pekos P. The use of fingerprint and marker compounds for identification and standardization of botanical drugs. J Drug Inform 1998; 32:497-512.

Liang YZ, Xie P, Chan K, J., Quality control of herbal medicines, Chromatogr B, 2004; 812: 53–70.

Marston A, Role of advances in chromatographic techniques in phytochemistry. Phytochem, 2002; 68: 2785-2797

Matthew C, Henry R. Supercritical fluid chromatography, Pressurized liquid extraction, and supercritical fluid extraction. Anal Chem 2006; 78: 3909.

McCouch S R, Kochert G, Yu Z H, Wang Z Y, Khush G S, Coffman W R, Tanksley S D. Molecular mapping of rice chromosomes. Theor Appl Genet. 1988;76: 815-829.

Mihalov, J. J., Marderosian, A. D., and Pierce, J. C. DNA identification of commercial ginseng samples, 2000

Mike Lee S, Edward Kerns H. LC/MS applications in drug development. Milestone Development Services, Pennington, New Jersey, 24 July 1999.

Mosihuzzaman M, Choudhary MI. Protocols on safety, efficacy, standardization, and documentation of herbal medicine. Pure Appl Chem 2008; 80(10):2195–2230.

Ong ES, Chemical assay of glycyrrhizin in medicinal plants by pressurized liquid extraction (PLE) with capillary zone electrophoresis (CZE). J Sep Sci, 2002; 25: 825-831

Patel PM, Patel NM, Goyal RK. Quality control of herbal products. The Indian Pharmacist 2006; 5(45):26-30.

Patil PS, Rajani S. An Advancement of Analytical Techniques in Herbal Research J.Adv.Sci.Res. 2010, 1(1); 08-14

Patil PS, Rajani S. An advancement of analytical techniques in herbal research. J Adv Sci Res 2010; 1(1):8-14.

Quality Control Methods for Medicinal Plant Materials, WHO, Geneva, 1996.

Ramakrishna W, Lagu M D, Gupta V S, Ranjekar P K., DNA fingerprinting in rice using oligonucleotide probes specific for simple repetitive DNA sequences., The Applied Genetics. 1994, 88,402-406

Rao Udaykumar B, Anna NP .Stability- indicating HPLC method for the determination of efavirenz in bulk drug and in pharmaceutical dosage form. Afr J Pharm Pharmacol 2009;3(12):643-650

Ratnaparkhe M. B., Gupta V. S., Venmurthy M. R. and Ranjekar P. K. Genetic fingerprinting of pigeonpea (Cajanus cajan (L.) Millsp.) and its wild relatives using RAPD markers, The. Applied Genetics, 1995, 91, 893–98.

Raya Luis Gomez, Hanne Gro Olsen, Frode Lingaas, Helge Klungland, Dag Inge Våge, Ingrid Olsaker, Seblewengel Bekele Talle, Monica Aasland, and Sigbjorn Lien. The Use of Genetic Markers to Measure Genomic Response to Selection in Livestock. Genetics. 2002,162, 1381-1388

Saravanan J, Shajan A, Joshi NH, Varatharajan R,Valliappan K. A simple and validated RP-HPLC method for the estimation of methylcobalamin in bulk and capsule dosage form. Int J Chem Pharm Sci 2010;1(2):323-324.

Schulz V, Hänsel R & Tyler VE Rational Phytotherapy. A Physicians' Guide to Herbal Medicine. 3rd edn. Springer-Verlag, Berlin. 1996.

Sharma AK, Gaurav SS, Balkrishna A. A rapid and simple scheme for the standardization of polyherbal drugs. Int J Green Pharm 2009; 3:134-140

Sharma, Handbook of Thin Layer Chromatography. Chromatographic Science Series, Marcel Dekker, Inc, New York Press; 55: 353-387.

Shaw P. C. and But P. P. Authentication of Panax species and their adulterants by random-primed polymerase chain reaction. Planta Med. 1995, 61, 466–69

Shikha S, Mishra N. Genetic markers - a cutting-edge technology in herbal drug research. J Chem Pharm Res 2009; 1:1-18.

Shrikumar S, Maheshwari U, Sughanti A, Ravi TK .WHO guidelines for standardization of herbal drugs. Pharminfo.net 2006; 2: 78-81.

Soni K, Naved T. HPTLC- Its applications in herbal drug industry. The Pharma Review 2010, 112-117

Srivastava S. et al. Genetic Markers - A Cutting-Edge Technology in Herbal Drug Research Journal of Chemical and Pharmaceutical Research2009, 1 (1): 1-18

Teo C.C., Tan S.N., Yong J.W. H., Hewb C. S., and Ong E. S.. Evaluation of the extraction efficiency of thermally labile bioactive compounds in Gastrodia elata Blume by pressurized hot water extraction and microwaveassisted extraction. J. Chromatogr. A 1182 : 2008 34– 40 .

Thoppil S.O., Cardoza R.M., and Amin P.D.. Stability indicating HPTLC determination of Trimetazidine as bulk drug and in pharmaceutical formulations, J. Pharm. Biomed. Anal 25(1): 20011, 5-20.

Waldesch FG, Konigswinter BS, Remagen HB (2003) Herbal medicinal products- Scientific and regulatory basis for development quality assurance and marketing authorization, published by medpharm stuttagart and CRC press, Washington DC, : 37-52.