A review on the Standardization of herbal medicines

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ABSTRACT

The world is witnessing an unprecedented growth in the usage of herbal products. India is a mother hub for natural herbs based science. Herbal drug technology is used for converting botanical materials into medicines, where standardization and quality control with proper integration of modern scientific techniques and traditional knowledge is important. For global harmonization WHO specific guidelines for the assessment of the safety, efficacy and quality of herbal medicines are of utmost importance. Standardization of drug means confirmation of its identity, quality and purity throughout all phases of its cycle. An overview covering the different techniques involved in standardization of crude/ finished compound drugs so far, e.g. macroscopic methods, microscopic methods, physical methods, chemical methods, biological methods.The chromatographic methods and comprehensive methods, such as fingerprint and multi-component quantification; hyphenated techniques, like HPLC-MS, GC-MS. and bhasma's standardization are also discussed.

KEY WORDS: WHO, Herbal medicine, Standardization, Quality control, DNA finger printing, Bhasma.

INTRODUCTION

The basic resources of medicines come from nature and they are used as medicaments from ancient time to present day. People around the world possess unique knowledge of the natural resources on which they depend, including tremendous botanical expertise. The traditional medicines cater about 85% of the world population for their health needs. It is essential to maintain safety, quality and efficacy of the plant and their products to avoid and serious health problems[1]. Indian healthcare consists of medical pluralism and ayurveda still remains dominant compared to modern medicine, particularly for treatment of a variety of chronic disease conditions[2]. WHO defines traditional medicine as including diverse health practises, approaches, knowledge and beliefs incorporating plant, animal and/or mineral based medicines, spiritual therapies, manual techniques and exercises applied singularly or in combination to maintain well being, as well as to treat, diagnose or prevent illness. 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. Herbal medicines are used very commonly in various health practices or therapies of Traditional Medicines like Chinese medicine, Ayurveda, Unani, Naturopathy, Osteopathy and Homeopathy[3].

Standardization

In recent years, there has been great demand for plant derived products in developed countries. These products are increasingly being sought out as medicinal products, nutraceuticals and cosmetics[4]. 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 quality control methods using a combination of classical and modern instrumental method of analysis. Standardization is an essential measurement for ensuring the quality control of the herbal drugs[5].

Standardization of herbal medicines is the process of prescribing a set of standards or inherent characteristics, constant parameters, definitive qualitative and quantitative values that carry an assurance of quality, efficacy, safety and reproducibility. It is the process of developing and agreeing upon technical standards. Specific standards are worked out by experimentation and observations, which would lead to the process of prescribing a set of characteristics exhibited by the particular medicines. Hence standardization is a tool in the quality control process[6]. American Herbal Product association defines: "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[7]. "Standardization" expression is used to describe all measures, which are taken during the manufacturing process and quality control leading to a reproducible quality. It also encompasses the entire field of study from birth of a plant to its clinical application. It also means adjusting the herbal drug preparation to a defined content of a constituent or a respectively by adding excipients or by mixing herbal drugs or herbal drug preparations[8]. "Evaluation" of a drug means confirmation of its identity and determination of its quality and purity and detection of its nature of adulteration[9].

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[10].

NEED OF STANDARDIZATION-

In olden days vaidyas used to treat patients on individual basis, and prepare drug according to the requirement of the patient. In almost all the traditional system of medicine, the quality control aspect has been considered from its inspection of its Rishis, Vaidyas and Hakims. Unlike in olden times where traditional practitioners prepared and tested the qualities of herbal medicines, the problem faced today are these of economics of industrial scale production, shelf life and distribution to long distances. These have necessitated development of modern and objective standards for evaluating the safety, quality and efficacy of these medicines. People are also becoming aware of the potency and side effect. To gain public trust and to bring herbal product into mainstream of today health care system, the researchers, the manufacturers and the regulatory agencies must apply rigorous scientific methodologies to ensure the quality and lot to lot consistency of the traditional herbal products[11]. Need of Quality control and standardization of herbal products can be summarized as follows-

1. When traditional medicines were developed technology and concept of standardization was quite different.
2. During past thousand years dynamic process of evolution may have changed the identity of plant material.
3. Due to commercialization, supply of genuine raw material has become a challenge.
4. Properties of botanicals may have undergone change due to time and environmental factors[12].

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[13]. Environmental conditions such as sunlight, rainfall, altitude, temperature, soil, storage conditions as well as different harvesting procedures, time and method of collection, manufacturing processes such as selecting, drying, purifying, extracting, and genetic variability can create substantial variability in product quality and in the concentration of plant chemicals within different products. Ecological conditions like insect feeding, microbial infections may affect secondary metabolites and in turn chemical composition of the plant. Also different parts of same plant (example roots, stem and leaves) contain different concentration of chemical constituents. At the same time diurnal variations (for example paclitaxel, opium alkaloids) and seasonal changes also account for variability in herbal medicines. The therapeutic or toxic components of plant vary depending on the part of the plant used as well as stages of ripeness[14]. Products from different manufacture vary considerably and it is not possible to control all the factors that affect the plants chemical composition[15 16].Due to complex nature and inherent

variability of the constituents of plant based drugs, it is difficult to establish quality control parameter and modern analytical technique are expected to help in circumventing this problem. Furthermore, the constituents responsible for the claimed therapeutic effects are frequently unknown or only partly explained. Most of the herbal formulations, especially the classical formulations of traditional medicine, are polyherbal. Many preparations are either liquid or semisolid. For such formulations it is very difficult to establish parameters for quality control. Even official standards are not available. The unique processing methods followed for the manufacture of these drugs turn the single drugs into very complex mixture, from which separation, identification and analysis of the components is very difficult.

Standardization of herbal products can be divided into two categories, first , an active constituents extract, where biochemical principles are known and have therapeutic values, and second, a marker extract, where the active principle is not known and a characteristic compound is used as marker to assess the presence of other therapeutic biochemical compounds[17]. Standardization has limitations because only isolated compounds are considered, ignoring the whole constituents of the herb, which may have synergistic or buffering activities to reduce the side effects.

Standardization of traditional medicine starts right from the collection of raw materials to the extreme clinical application. In case of traditional medicine, the therapeutic efficacy is a total effect of its chemical constituents. So, the quality and purity refers to the total profile of the drug rather than any of its character. Therefore, a multidimensional approach is essential for standardization of traditional medicine. This multidimensional approach should cover every minute aspect of drug specifically the name, botanical source, geographical source, organo-leptic, morphological, anatomical, physical, chemical and biological activities. 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[13]. The authenticity, quality and purity of herbal drugs are established by references given in pharmacopoeia. These documents publish traditional and standardized therapeutic uses of herbs and provide a foundation for clinical practice. Monographs consist of a description of the herb, including botanical information, laboratory analysis, therapeutic indications and drug interactions. The pharmacopoeia prescribes (numerical value) like structural, analytical, physical standards for the drugs[18].

CONVENTIONAL METHODS FOR STANDARDIZATION OF CRUDE DRUG-

Standardization of herbal raw drug includes passport data of raw plant drugs. It includes medico- botanical survey, identification, botanical authentification, macroscopic, examination. Testing of drugs as per approved Pharmacopoeial testing protocol- Fully pharmacognostical profile, Identification by various chromatographic techniques, Assessment of purity by physico-chemical profile, Assessment of strength by active marker or assay estimation and Safety by heavy metal profiling, microbiological limit test analysis, aflatoxins analysis, pesticides residue and biological activity[19]. 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 [5 20]. The phytochemical evaluation for standardization purpose includes the following- Preliminary testing for the presence of different chemical groups, quantification of chemical groups of interest (e.g., total alkaloids, total phenolics, total triterpenic acids, total tannins), establishment of fingerprint profiles, multiple marker-based fingerprint profiles and quantification of important chemical constituents[21].

STANDARDIZATION OF HERBAL/ POLYHERBAL FORMULATION

The herbal formulation in general can be standardized as to formulate the medicament using raw material collected from different localities and a comparative chemical efficacy of different batches of formulation are to be observed. The preparations with better clinical efficacy are to be selected. All the routine physical, chemical and pharmacological parameters are checked for all the batches in order to select the final finished product and to validate the whole manufacturing process. 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[22]. Standardization minimizes batch to batch variation; assure safety, efficacy, quality and acceptability of the polyherbal formulations[23].

Standardization of herbal formulation requires implementation of Good Manufacturing Practices[24]. 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. Heavy metals contaminations, Good Agricultural Practices (GAP) in herbal drug standardization are equally important[25].

In indigenous/traditional system of medicine, the drugs are primarily dispensed as water decoction or ethanol extract. Thus the medicinal plant parts should be authentic and free from harmful material like pesticides, heavy metals, microbial, radioactive contamination, etc. The medicinal plant is subjected to a single solvent extraction once or repeatedly, or water decoction or as described in ancient texts. The extract should then be checked for indicated biological activity in an experimental animal model. The bioactive extract should be standardized on the basis of active principle or major compound(s) along with fingerprints. The next important step is stabilization of the bioactive extract with a minimum shelf life of over a year. The stabilized bioactive extract should undergo regulatory or limited safety studies. Determination of the probable mode of action will explain the therapeutic profile. The safe and stable herbal extract may be marketed if its therapeutic use is well documented in indigenous systems of medicine, as also viewed by WHO. A limited clinical trial to establish its therapeutic potential would promote clinical use. The herbal medicines developed in this mode should be dispensed as prescription drugs or even OTC products depending upon disease consideration.[26 27]

WHO GUIDELINES FOR QUALITY STANDARDIZED HERBAL FORMULATIONS

1) Quality control of crude drugs material, plant preparations and finished products.

2) Stability assessment and shelf life

3. Safety assessment; documentation of safety based on experience or toxicological studies.

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

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

Generally, all medicines , whether they are synthetic or of plant origin, should fulfil the basic requirement of being safe and effective[28 29]. The term 'herbal drugs' denotes plants or plant parts that have been converted into phytopharmaceuticals by means of simple processes involving harvesting, drying and storage[30].

1. Quality Control Of Herbal Drugs -

Quality control is a term that refers to processes involved in maintaining the quality and validity of a manufactured product. In general, quality control is based on three important pharmacopeial aspects -

a. Identity or authenticity- it should have one herb
b. Purity – it should not have any contaminant other than herb
c. Assay or Content -the active constituents should be within the defined limits.

Identity can be achieved by macro and microscopical examinations. In addition to this identity tests, which include simple chemical tests, eg.colour or precipitation and chromatographic tests are also necessary. These chemical and chromatographic tests help to provide batch to batch comparability and the chromatogram may be used as a 'fingerprint' for the herbal ingredient by demonstrating the profile of some common plant constituents such as flavonoids, alkaloids and terpenes. To prove identity and purity, criteria such as type of preparation, sensory properties, physical constants, adulteration, contaminants, moisture, ash content, and solvent residues have to be checked. Voucher specimens are reliable reference sources. Outbreaks of diseases among plants may result in changes to the physical appearance of the plant and lead to incorrect identification[31 32] Purity is closely linked with safe use of drugs and deals with factors such as ash values, contaminants (e.g. foreign matter in the form of other herbs), and heavy metals. However, due to the application of improved analytical methods, modern purity evaluation also includes microbial contamination, aflatoxins, radioactivity, and pesticide residues. Analytical methods such as photometric analysis, Thin layer chromatography (TLC), High performance liquid chromatography (HPLC), High performance thin layer chromatography (HPTLC), and Gas chromatography (GC) can be employed in order to establish the constant composition of herbal preparations. Depending on whether the active principles of the preparation are known or unknown, different concepts such as "normalization versus standardization" have to be applied in order to establish relevant criteria for uniformity. Content or assay is the most difficult area of quality control to perform, since in most herbal drugs the active constituents are unknown. Sometimes markers can be used. In all other cases, where no active constituents or marker can be defined for the herbal drug, the percentage extractable matter with a solvent may be used as a form of assay, an approach often seen in pharmacopeia[33 34].A special form of assay is the determination of essential oils by steam distillation. When active constituents (e.g. sennosides in senna) or markers (e.g. alkydamides in Echinacea) are known, a vast array of modern chemical analytical methods such as ultraviolet/visible spectroscopy(UV/VIS), TLC, HPLC, HPTLC, GC, mass spectrometry, or a combination of GC and MS(GC/MS), can be employed[35].

2. Stability Assessment And Shelf Life

Prolonged and apparently uneventful use of a substance usually offers testimony of its safety. In a few instances, however, investigation of the potential toxicity of naturally occurring substances widely used as ingredients in these preparations has revealed previously unsuspected potential for systematic toxicity, carcinogenicity and

teratogenicity. Regulatory authorities need to be quickly and reliably informed of these findings. They should also have the authority to respond promptly to such alerts, either by withdrawing or varying the licences of registered products containing suspect substances, or by rescheduling the substances to limit their use to medical prescription.

AssesementOf Quality

All procedures should be in accordance with good manufacturing practices.

Crude Plant Material

The botanical definition, including genus, species and authority, description, part of the plant, active and characteristics constituents should be specified and, if possible content limits should be defined. Foreign matter, impurities and microbial content should be defined or limited. Voucher specimens, representing each lot of plant material processed, should be authenticated by a qualified botanist and should be stored for at least a 10-year period. A lot number should be assigned and this should appear on the product label.

Plant Preparations

The manufacturing procedure should be described in detail. If other substances are added during manufacture in order to adjust the plant preparation to a certain level of active or characteristics constituents or for any other purpose, the added substances should be mentioned in the manufacturing procedures. A method for identification and, where possible, assay of the plant preparation should be added. If identification of an active principle is not possible, it should be sufficient to identify a characteristic substance or mixture of substances to ensure consistent quality of the preparation.

Finished Product

The manufacturing procedure and formula, including the amount of excipients, should be described in detail. A finished product specification should be defined to ensure consistent quality of the product. The finished product should comply with general requirements for particular dosage forms.

Stability

The physical and chemical stability of the product in the container in which it is to be marketed should be tested under defined storage conditions and the shelf-life should be established.

3. Safety Assessment:

Herbal medicines are generally regarded as safe based on their long-standing use in various cultures. However, there are case reports of serious adverse events after administration of herbal products. In a lot of cases, the toxicity has been traced to contaminants and adulteration. However, some of the plants used in herbal medicines can also be highly toxic. As a whole, herbal medicines can have a risk of adverse effects and drug-drug and drug-food interactions if not properly assessed.

Assessment of the safety of herbal products, therefore, is the first priority in herbal research.

These are various approaches to the evaluation of safety of herbal medicines. The toxic effects of herbal preparation may be attributed mainly to the following: Inherent toxicity of plant constituents and ingredients and Manufacturing malpractice and contamination.

Evaluation of the toxic effects of plant constituents of herbal formulation requires detailed phyto-chemical and pharmacological studies. It is, however, safe to assume that, based on human experiences in various cultures, the use of toxic plant ingredients has already been largely eliminated and recent reports of toxicity could largely ne due to misidentification and overdosing of certain constituents[36]. Adulteration of botanical preparations is another important issue. Due to over exploitation of certain plants, habitat loss and fragmentation of the forest, many medicinal plants have reached to the level of the endangered or rare species. These and many other factors (like cost of raw material) cause problem for availability of genuine drug, which encourages the adulteration of plant by substitution with inferior commercial varieties, artificially manufactured substances, exhausted drugs or cheaper plant or by another vegetative part[37]. Several reports suggest that many herbal products contain undisclosed pharmaceuticals and heavy metals[38]. The intentional use of pharmaceutical adulterant is possible. Agrochemicals are used to protect the plant from the crude plant material. More over mechanism of action, pharmacokinetics and drug-drug interactions of many herbs are still in infancy. At the same time growing number of reports about fatal or adverse effects of herbal preparations intensifies need for national regulation and registration of herbal medicines and establishment of safety monitoring. Clinicians should not prescribe or recommend herbal remedies without well-established efficacy as if they were medications that had been proved effective by rigorous study[39].

Assessment Of Toxicity

Toxicity investigation will also be required because the analysis alone is unlikely to reveal the contributions to toxicity itself. In assessing toxicity of an herbal medicine, the dose chosen is very important[40].

Toxicity assessment involves one or more of the following techniques- In vivo techniques, in vitro techniques, cell line techniques, micro- array and other modern technique, standardization and techniques to adequately model toxicity.

4. Assessment Of Efficacy

Herbal medicines are inherently different from conventional pharmacological treatments, but presently there is no way to assess their efficacy other than by currently used conventional clinical trial methodologies, in which efficacy is conventionally assessed by clinical, laboratory, or diagnostic outcomes: Clinical outcomes include parameters such as improved morbidity, reduced pain or discomfort, improved appetite and weight gain, reduction of blood pressure, reduction of tumor size or extent, and improved quality of life. Laboratory /other diagnostic outcomes include parameters such as reduction of blood glucose, improvement of hemoglobin status, reduction of opacity as measured by radiological or imaging techniques, and improvement in electrocardiogram (ECG) findings.

Implementation of a standardized approach for the herbal practitioners and collection of the prospective data necessarily creates an interventional design which, if planned properly, may closely resemble single-blind randomized trials. Even if it differs from double-blind randomized trials in the degree of rigor, the design may be the optimum, both biologically and economically, for rapid evaluation of herbal products. Standardization, however, may sometimes be incompatible with the existing legislative framework and caution is needed regarding the ethical implications of such studies. Although randomized clinical trials (with double blind trials as the gold standard) are relatively difficult to be implemented in the case of herbal medicine, they are not ruled out per se in assessing the efficacy of these products. Data from case series studies may provide sufficient scientific and ethical validity to conduct such trials, but acceptance of this protocol needs a paradigm change in the methodoly of drug evaluation as understood in conventional medicine.

Standardization and Quality control of herbal drugs involve wide array of scientific investigations, which include physical, chemical and biological evaluation employing various analytical method and tools.

Physical Evaluation- Each monograph contains detailed botanical, macroscopic and microscopic descriptions with detailed illustrations and photographic images which provide visual documentation of accurately identified material. A microscopic analysis assures the identity of the material and as an initial screening test for impurities.

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

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

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

The most important step in the development of analytical methods for botanical and herbal preparations is sample preparation. The basic operation includes steps such as pre- washing, drying of plant materials or freeze-drying and grinding, to obtain a homogenous sample and often improving the kinetics of extraction of the constituents. In the pharmacopoeial monographs, method such as sonication, heating under reflux, Soxhlet extraction, and others are commonly used[4142] However, such methods can be time-consuming, require the use of a large amount of organic solvent, and may have lower extraction efficiencies. New methods are continuously being sought to address this issue. As target compounds may be polar or nonpolar and even thermally labile, the suitability of the methods of extraction must be considered. To reduce or eliminate the use of organic solvents and improve the extraction processes, newer sample preparation methods, such as microwave-assisted extraction (MAE), supercritical fluid extraction (SFE), and accelerated solvent extraction (ASE) or pressurized liquid extraction (PLE) have been introduced for the extraction of targeted constituents present in plant materials.

Chromatogarphy-Separation of individual components from the herbal mixture is the key step to enable identification and bioactivity evaluation. Chromatography is a powerful analytical method suitable for the separation and quantitative determination of a considerable number of compounds, even from a complex matrix. These include paper chromatography (PC), thin-layer chromatography (TLC), gas chromatography (GC), HPLC, and capillary electrophoresis (CE).

TLC is used extensively in the phytochemical evaluation of herbal drugs because It enables rapid analysis of herbal extracts with minimum sample clean-up requirement, It provides qualitative and semi quantitative information of the resolved compounds. In TLC fingerprinting, the data that can be recorded using a high-performance TLC (HPTLC) scanner includes the chromatogram, retardation factor (Rf) values, the color of the separated bands, their absorption spectra, λ max and shoulder inflection/s of all the resolved bands. All of these, together with the profiles on derivatization with different reagents, represent the TLC fingerprint profile of the

sample. 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[43 44]. There are basically two types of preparative HPLC: low pressure HPLC (typically under 5 bar) and high pressure HPLC (pressure >20 bar)[45]. HPTLC has been investigated for simultaneous assay of several components in a multi-component formulation[46]. It has been well reported that several samples can be run simultaneously by use of a smaller quantity of mobile phase than in HPLC[47]. 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[48]. LC-MS has become method of choice in many stages of drug development[49]. The chemical standardization of an aqueous extract of the mixture of the herbs provided chemical compounds serving as reference markers using LC-MS[50]. 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 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[51].Gas chromatographic equipment can easily interfaced with rapid scan mass spectrometer of various types. The flow rate of the capillary column is generally low but enough that the column. Output can easily fed directly into ionization chamber of MS. In this the simplest mass detector in GC is the Ion Trap Detector46[52]. 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[53].

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[54].

UV absorption has been the most commonly used detection method for the preliminary identification of the separated components. However, various other detectors, such as fluorescence (FD), flame ionization (FID), electron capture (ECD), refractive index (RI), and most recently, evaporative light scattering (ELSD), are also available for specific cases. Most of these detection methods allow the quantification of chemical compounds present in plant material or herbal product. The availability of high-speed computing and the appropriate software allows detection by using mass spectrometry (MS). This method not only allows the detection of component peaks of a mixture separated by chromatography but also in combination with UV (using a photodiode array detector), multistage MS and nuclear magnetic resonance spectrometry (LC–UV–MS–NMR), allows its molecular characterization[55 56]. More recently, NMR metabonomics, in combination with chemometrics, especially principal component analysis (PCA) and simulated independent modeling of class analogy (SIMCA) algorithms, has been recognized as a very powerful tool to classify samples according to their total chemical composition. The resolution of high-field NMR can provide information in the orders of magnitude higher than of other fingerprinting technologies such as usual NMR spectrometry or HPLC. This is a nonreductuive fingerprinting method of the total chemical composition of samples[57 58 59 60].

The presence of toxic metals is also one of the parameters included in pharmacopoeias. The tool primarily used to detect and quantify the elements in most analyses is based on atomic absorption spectrometry (AAS). Currently, there have been a number of instruments developed based on the same principle, such as inductively coupled plasma–optical emission spectrometry (ICP–OES). Detection and quantification based on mass spectrometry has also been available using inductively coupled plasma–mass spectrometry (ICP–MS) [61].

STANDARDIZATION OF BHASMAS

Bhasmas are made from metals like zinc, lead, gold, silver, tin, copper, metal mixtures and alloys as also from gems, coral and mica and some other minerals, etc. These are formed by calcinations of the parents' substances like minerals, etc. in a rigorous, prescribed manner after it has been appropriately purified and emasculated with herbal juices or minerals[62]. Bhasma are widely recommended for treatment of a variety of chronic ailments and are taken along with milk, butter, honey, or ghee to eliminate the harmful effects of metals and enhancing their biocompatibility in the body[63].

The Ayurvedic Formulary of India lists more than one method of preparation of the bhasmas of some metals while the Drug and Cosmetics Act lists several traditional texts that a vaidya can refer to for the preparation of bhasma[64]. A standardization of process and the end product both is called for Pharmacopoeial standards have been published by the Government of India for a large number of single drugs of plant origin and the work on classical composite formulations is being carried in several human laboratories. The CCRAS has developed a technique called phase spot test for identification and quality assessment of bhasmas[65]. In recent years, sophisticated test equipments and techniques of data analysis have been put to fruitful use for the

physicochemical characterization of the bhasmas. These studies also explored intensively the different bhasma preparation methods of the same metal, chemical nature and crystalline structure of the intermediates and the final products. These have involved Atomic Absorption Spectrophotometry (AAS), flame photometry, Inductively Coupled Plasma Atomic Emission Spectrometry (ICP- AES), X-ray diffraction (XRD) analysis and pHmetry, etc. The AAS uses the property of atoms to absorb certain wavelengths of electromagnetic radiation. The amount of light absorbed enables one a quantitative estimate of absorbing element. The ICP- AES uses plasma (e.g. inductively coupled plasma) to generate excited atoms. These atoms emit electromagnetic radiation at a wavelength that is characteristic of a particular element. From a measure of the intensity of this emission one can quantify the concentration of the element present in a sample. The techniques involving X- ray diffraction analysis reveal information about the crystallographic structure (arrangement of molecules in a crystal) to know whether the particles are crystalline or amorphous and the chemical composition and physical properties of materials. From results on particle size distribution and crystal structure, one can determine how well the bhasma process has proceeded. With such equipment in hand, it should be possible to standardize and freeze the SOPs for bhasma preparations. A ten point"s protocol has been suggested for the standardization of bhasmas and the process of their preparations[66].A standardization programme should also distinguish between bhasmas of metals made of either herbs and non-toxic minerals and those with toxic substances.

DNA FINGERPRINTING TECHNIQUE

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[67]. 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[68]. 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[69].DNA markers are helpful to identity cells, individuals or species as they can be used to produce normal, functioning proteins to replace defective ones. Moreover, these markers help in treatment of various diseases and help in distinguishing the genuine herb from adulterated drug. Cannabis sativa and Arabidopsis thaliana L. Heyne have been differentiated from their adulterated species by using ISSR markers[70].

ROLE OF GENETIC MARKERS IN THE STANDARDIZATION OF HERBAL DRUGS

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 categorised[71]. RAPD-based molecular markers have been found to be useful in differentiating different accessions of neem collected from different geographical regions[72]. 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, pearlmilletetc is being carried out extensively[73]. 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) [74].

RAPD markers have been successively used for selection of micropropogated plants of Piper longum for conservation[75 76].

CONCLUSION

The Indian herbal industry is growing in a tremendous rate. With the tremendous increase in traditional herbal therapy several concerns regarding the safety and quality of herbal medicines have also been observed. There is need for more advanced techniques of standardization. The advancement of analytical techniques will serve as a rapid and specific tool in the herbal research, thereby, allowing the manufacturers to set quality standards and specifications so as to seek marketing approval from regulatory authorities for therapeutic efficacy, safety and shelf- life of herbal drugs. The national health authorities should ensure that all herbal pharmaceutical product subject to their control are in conformity with quality, safety, efficacy and all premises and practises employed the manufacturing and distribution of these product comply with GMP standards so as to ensure the continued

conformity of the products with these requirements until such time as they are delivered to the end user. Quality control of herbal medicines has not only to establish reasonable analytical methods for analyzing the active constituents in herbal medicines, but many other factors should be concerned, such as pesticides residue, aflatoxins content, the heavy metals contamination, good agricultural practice (GAP), good manufacturing practice (GMP), etc. There is need for development of techniques which includes both traditional methods of evaluation and modern methods of evaluation. This will improve the quality of the drug and also motivates the practitioners to get more involved in the standardization process.

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